VOLUME 47 JUNE 1961 NUMBER 3

The Journal of PARASITOLOGY

FOUNDED BY HENRY BALDWIN WARD

OFFICIAL ORGAN OF THE
AMERICAN SOCIETY OF PARASITOLOGISTS

EDITOR
ELERY R. BECKER

ASSOCIATE EDITORS
HAROLD W. MANTER
HERBERT T. DALMAT

ASSISTANT EDITORS

RAYMOND M. CABLE
QUENTIN M. GEIMAN
DOMINIC L. DEGIUSTI
KENNETH C. KATES
GLEN M. KOHLS
GEORGE R. LA RUE
ROBERT M. LEWERT
R. BARCLAY McGHEE
ALLEN McINTOSH
CLARK P. READ
RALPH E. THORSON
PAUL P. WEINSTEIN

THE JOURNAL OF PARASITOLOGY

The Journal of Parasitology, published bi-monthly, is both the official organ of the American Society of Parasitologists and a medium for the publication of the results of research in the field of animal parasitology.

Manuscripts intended for publication may be sent to the Editor, Elery R. Becker, Communicable Disease Center, Public Health Service, 702 E. Adams St., Phoenix 34, Arizona, or any other member of the Editorial Board, with the understanding that they are to be published exclusively in the Journal unless otherwise arranged in advance. All papers received will be submitted to competent reviewers, either members of the Board or other persons with special qualifications. Authors will be notified when their papers have been accepted for publication. Correspondence regarding manuscripts should be addressed to the Editor.

Manuscripts should be typewritten, double-spaced, with wide margins at sides, top and bottom, and with tables and illustrations in finished form. Sentences should be concise and clear, and the text should be as condensed as possible because of mounting costs of publication. Title, author, address, etc., as well as footnotes, should appear on a separate sheet. Footnotes should be avoided whenever possible. Each table requires a separate sheet. Tables should be simple, since complex ones are difficult to inspect. Every effort should be made to limit their width so that they can be accommodated by the 16-pica column (2⅝ inches).

Tabulation should be avoided when the material can as well be summarized in the text. Extensive duplication or triplication of data in text, tables and graphs should also be avoided. Sharp, contrasty, glossy photographs of plates that will fit into a 9- × 12-inch envelope should be sent instead of the originals, since the latter have often been badly damaged in the mails. Plates should be arranged with a 5- × 8-inch area or multiple thereof. Whenever possible, figures should be drawn so that when reduced they can be accommodated by one of the 16-pica columns. Legends for figures should be typed on a page separate from the text and illustrations. Greek characters and symbols should be avoided on account of cost.

Bibliography should include only references cited in the text, and its style should conform to that in recent issues of the Journal. Suggested abbreviations for names of journals have been published (J. Parasit. 46(3): 308, 316, 326), as well as a note on bibliographic style (J. Parasit. 45(6): 6).

Acceptability of a paper is determined not by its length, but by the amount and character of new information included and the form in which it is presented. Long articles and those with unusually large number of plates can be published only with the financial cooperation of the author.

Reprints may be purchased at cost from the printer and are to be ordered from the printer immediately after receipt of galley proof. The editorial office has neither responsibility nor concern with reprints. The galley proof is returned to the Editor. Claims for copies lost in the mails must be received by the Treasurer within 60 days of the mailing date. Delivery overseas must be at the subscriber's risk. Change of address must be received by the Treasurer 2 weeks prior to mailing date.

The subscription price for the Journal is $15.75 domestic, $16.50 foreign. Orders should be addressed to the Treasurer, Martin J. Ulmer, Department of Zoology, Iowa State University, Ames, Iowa.

Correspondence concerning back issues should be addressed to the Custodian, Wilford S. Bailey, School of Veterinary Medicine, Auburn Polytechnic Institute, Auburn, Alabama.

Persons interested in membership in the American Society of Parasitologists may obtain application blanks from the Secretary. A year's subscription to the Journal of Parasitology is included in the annual dues ($15.00).

Second-class postage paid at Lancaster, Pa. Printed at 10 McGovern Ave., Lancaster, Pa.
© American Society of Parasitologists 1961

The Journal of Parasitology

Volume 47 JUNE 1961 Number 3

THE IN VITRO CULTIVATION OF TAPEWORMS. I. GROWTH OF *HYMENOLEPIS DIMINUTA* (CESTODA: CYCLOPHYLLIDEA)

ALLEN K. BERNTZEN

Portland State College, Portland, Oregon*

Valuable contributions have been made to the field of parasitology by the development of in vitro culture methods. Successful in vitro culture of cestodes has been achieved primarily with pseudophyllideans, as shown by the outstanding studies of Smyth (1955, 1956, 1958) who cultured *Schistocephalus, Ligula,* and *Diphyllobothrium,* and also the most recent work of Mueller (1958, 1959) who has cultured *Spirometra mansonoides.*

To the author's knowledge, the only reports on successful in vitro culture of adult cyclophyllideans is that of Schiller, Read, and Rothman (1959) who reported a 30-fold increase in growth of *Hymenolepis diminuta.* As previously reported (Berntzen, 1960), the cestode *Hymenolepis diminuta* has been successfully cultivated in vitro from excysted larvae to adults having proglottids with developing oncospheres. This method, employing a continuous flow principle, will be described in detail.

The author wishes to express his appreciation to Dr. Ralph Macy, Portland State College, Portland Oregon, who by his teaching stimulated the work reported herewith; to William P. Temple, Portland, Oregon, for his assistance in the designing of the culture apparatus; to Bonnie S. Berntzen for her assistance and encouragement throughout this work; and to Dr. Marietta Voge for her enthusiasm and assistance in evaluating the method, and for her help in the preparation of this report.

MATERIALS AND METHODS

Beetles infected with *H. diminuta* cysticercoids were obtained from the Carolina Biological Supply Company. The cysticercoids were dissected free of the beetle tissue in Parker's tyrode solution and pipetted into petri dishes containing sterile Tyrode's solution. After two changes of Tyrode's they were placed in an incubator at 37 C until they excysted. If excystation had not begun within 30 minutes, the pH of the Tyrode's solution was raised from 7.8 by the gradual addition of small amounts of 0.1 normal sodium hydroxide or 1.0 normal sodium bicarbonate until excystment. As a rule, excystment took place at pH 8.0 to 9.0.

A simple, but effective method for sterilization of excysted larvae is by continuous flow of sterile Tyrode's solution over the larvae. This was achieved by designing a glass chamber which allowed maintenance of sterile conditions. Figure 1 illustrates the apparatus used in the washing procedure. The larvae are placed in the specimen holder and the unit assembled, the fluid supply valve turned on, and the fluid exhaust adjusted to the volume of the fluid supply. By this procedure the larvae are exposed to steady, gentle agitation and a continuous change of the sterile solution. At first large volumes (10 to 15 liters) of Tyrode's solution were passed over the larvae; at present 1 to 2 liters are used with satisfactory results. After sterilization the larvae are placed in petri dishes with medium until inoculation into the culture tube. All culture tubes are lined with strips of Whatman No. 3 filter paper.

The culture apparatus operates on a continuous flow principle. It is devised to maintain a continuous flow of medium over the worms at a controlled rate. It allows for the collection of medium which presumably has been altered by the worms metabolic processes. The collected medium can be removed at any time interval for analysis without disturbing the culture. Temperature is controlled to ± 0.5 C. Figure 2 illustrates and describes the culture apparatus.

It should be noted (1) that the culture tube (h) lies at an angle of approximately 10°, and (2) that the air interruptor (u) serves for air circulation and assists in maintaining uniform temperature.

After the excysted larvae of *H. diminuta* are inoculated into the culture tubes, the culture apparatus is assembled, and the medium supply is adjusted to deliver 20 drops per minute. The culture is maintained at 37 ± 0.5 C.

Several types of media were tried until a medium in which the tapeworms would grow was successfully produced. The composition of this medium is given in table I. Extracts, hormones, and various other organic materials are added to a base medium of whole human blood plasma and Tyrode's solution in the order given in table I.

Received for publication December 29, 1960.

* Present address: Department of Infectious Diseases, School of Medicine, University of California, Los Angeles.

This project was aided by a research grant from Portland State College.

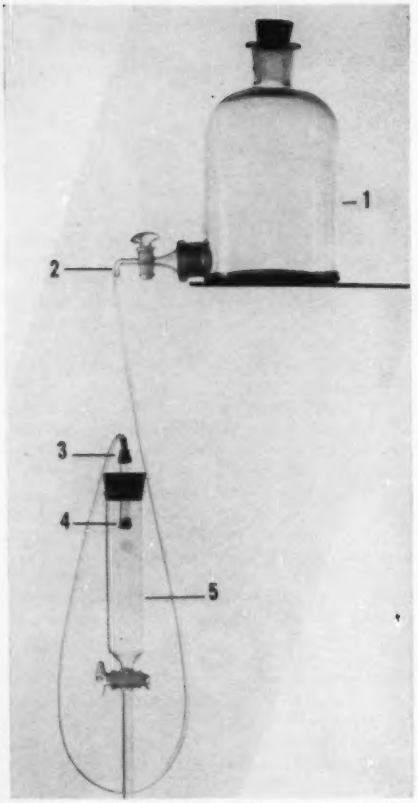


FIGURE 1. Apparatus used for washing larvae. 1, fluid supply bottle; 2, supply valve, with one-eighth inch inside diameter Tygon tubing; 3, vial cap adapting tubing to specimen holder; 4, silk mesh covering end of specimen holder; 5, separatory funnel with stop cock (fluid exhaust).

In preparing the medium it is essential that glassware and all equipment be acid cleaned and well rinsed with distilled water, followed by autoclaving at 20 lbs for 30 minutes. Aseptic technique is followed throughout the medium preparation. Tyrode's solution is prepared according to Parker (1950, pp. 77–79). The final Tyrode's solution should have a pH range of 7.4 to 7.8. The completed Tyrode's solution is sterilized by passing it through a Berkefeld filter into sterile bottles and stored under refrigeration.

Whole human blood, which has been heparinized, and checked for bacterial contamination, is obtained and stored under refrigeration. The blood cells are allowed to settle and the plasma is drawn and warmed to room temperature. This plasma is mixed 1:1 with Tyrode's solution and allowed to stand until a gel is formed. This gel is broken by violent shaking, and the resulting liquid filtered through Watman No. 1 filter paper. The resulting filtrate of the plasma and Tyrode's mixture constitutes the base medium. Chick embryo extract is prepared by homogenizing 18- to 20-day embryos with 50 cc Tyrode's solution per embryo. Homogenation is followed by centrifugation for 10 minutes at 2500 rpm in sterile capped tubes. To prepare the stock medium, the organic materials and extracts are added in the amounts and order designated in table I per 1000 cc base medium. The base medium plus the organic substances and extracts represent the stock solution which is diluted 1:1 with Tyrode's solution just before use.

TABLE I. *Organic materials* added to 1,000 cc of base medium.*

Name	Amount used
DL cysteine hydrochloride**	10 cc of 0.10 M
DL thyroxine**	25 cc of 0.025 M
Thiamin chloride**	50 cc of 0.05 M
Nicotinic acid**	50 cc of 0.05 M
Methyl testosterone**	100 cc of 0.025 M
Riboflavin**	25 cc of 0.10 M
Bacto-Yeast Extract** (B-127)	100 cc of 0.5% solution
Chick embryo extract	100 cc of extract

* Obtained from Nutritional Biochemicals Corporation.

** Each of the above was mixed in sterile pyrogen-free water, filtered through a Berkefeld filter and refrigerated until use.

Worms were removed on the 3d, 4th, 7th, 10th, 12th, and 15th day of culture for length measurements. The experiment was terminated on the 15th day when specimens were fixed in hot acetic acid and formalin and stained with hematoxylin or by the method of Tahmisian (1945).

RESULTS

The cestode *H. diminuta* was successfully cultured in vitro from the cysticercoid stage in beetles to adults which contained developing oncospheres. A series of three consecutive experiments, utilizing the techniques and medium described here, yielded positive results. In each case the larvae were cultured to adults which had proglottids containing developing oncospheres. In the first two experiments no attempt was made to establish a record of growth rate. In the third experiment total length measurements were taken on days 3, 4, 7, 10, 12, and 15; these are shown in table II. Four culture tubes were utilized and a total of 16 worms was measured on days indicated. The rate and range of total length change is graphed in figure 3. There is little change in length between the 3rd and 4th day; however, between the 4th and 15th day there appears a sharp daily increase in total length. Figure 4 illustrates worms grown for 7 days and for 15 days.

Stained specimens of a 15-day culture

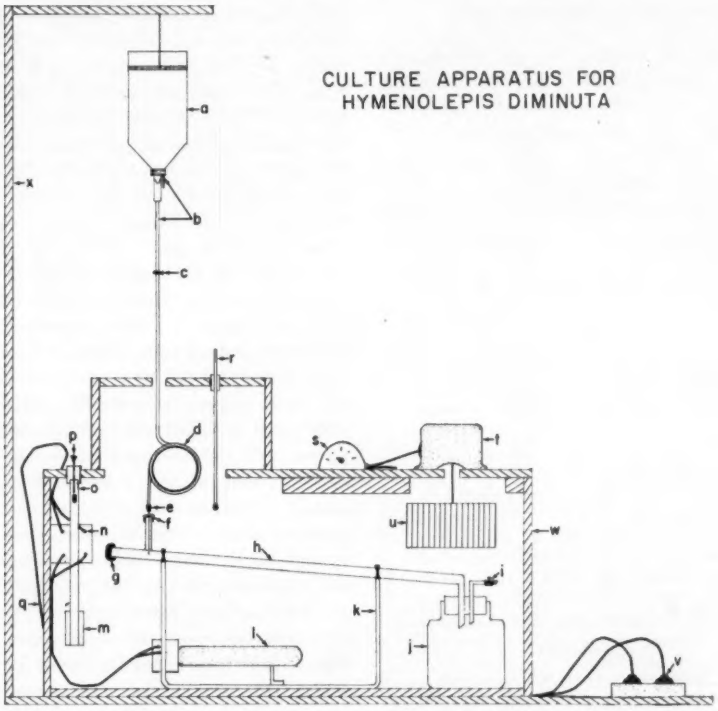


FIGURE 2. a, 500-cc Abbott bottle, medium supply container; b, Abbott intravenous drip set with tubing; c, screw clamp, used to adjust rate of medium flow; d, wire core with medium supply tubing coiled around it, used to pre-heat medium before entering culture tube; e, 15-gauge needle; f, vial cap over medium inlet of culture tube; g, vial cap sealing innoculation opening of culture tube; h, culture tube, made of 15-mm diameter pyrex tubing 24 in. long with an inlet side arm 3 mm diameter by 3 in. long, and an outlet arm of 3 mm diameter 4 in. long (The main body of the tube is lined with Watman No. 3 filter paper); i, air exhaust tube with screw clamp, also used in regulating medium flow; j, medium collecting bottle with stopper; k, metal frame with clamps to support the culture tubes; l, 40-watt aluminum coated show case bulb, with receptacle; m, 70-watt wire heat coil; n, heat unit junction box to thermostat; o, thermostat; p, temperature control; q, power supply line; r, thermometer; s, speed control of air interruptor; t, air interruptor motor; u, air interruptor; v, electrical power source; w, wood case housing culture unit; x, medium supply support.

showed normal segmentation and scolices. Mature segments contained well developed gonads and could not be differentiated from worms grown in rats. Development of the uterus and oncospheres in the semi-gravid region of the strobila was normal. Oncospheres had not reached the stage of hooklet formation. About 50 or more semi-gravid segments were present in each worm.

TABLE II. *Length ranges and averages of* H. diminuta *at different periods of culture.**

Day of culture	Number of animals	Total length in centimeters	
		Range	Average
3	16	1.0 – 0.4	0.60
4	16	1.0 – 0.5	0.69
7	16	2.5 – 1.0	1.70
10	16	9.0 – 3.0	4.80
12	16	18.0 – 6.0	13.00
15	16	25.0 – 15.0	20.80

* Composite table of one experiment utilizing four worms in each tube. Medium was the same in all tubes.

DISCUSSION

The method described was designed especially for the in vitro culture of cestodes. Standard approaches of culture were discarded and thought to be inadequate for the following reasons. Cestodes live normally under specialized conditions. They do not "float" freely in a

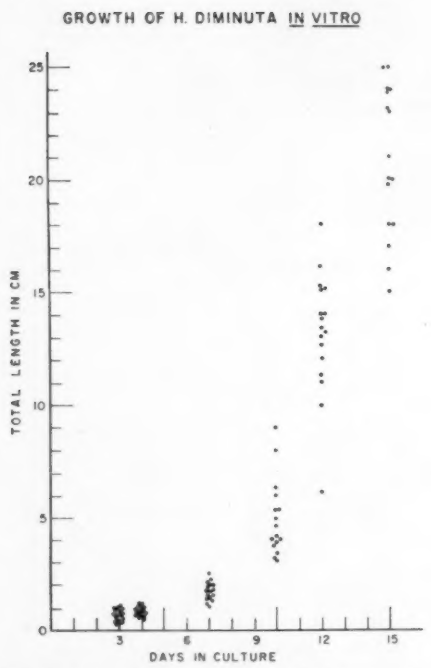


FIGURE 3. Length attained by *Hymenolepis diminuta* at different days of culture; each dot represents one worm.

liquid environment, but are instead firmly attached and can either move their strobilae or apply them to a surface. Temperature, pH, and "atmosphere" are relatively constant. Food supply is continually moving by in a semi-solid state, and metabolic waste is eliminated continually. It is with these thoughts in mind that the apparatus and method were devised, so as to imitate as closely as possible the conditions encountered in the gut of a host animal. Furthermore, it was thought that the physical aspects of the environment may have profound effects on the successful maintenance of the organism in vitro. This is suggested by the fact that larvae in flasks, tubes, or petri dishes with the same medium and temperature have failed to grow and to differentiate, whereas larvae from the same group, innoculated into the culture apparatus designed for continuous flow, have grown and differentiated successfully.

Even though there exists in this method of culture a multitude of problems and details of manipulation, it does in effect work. Whether or not all the steps and ingredients described are essential for successful growth of the worms has not yet been determined.

Concerning the method of medium preparation, it was found that the order in which the ingredients were mixed together is of importance. For example, the base medium of whole human blood plasma and Tyrode's solution must form a gel and then be broken up before any other ingredient is added. The medium is an

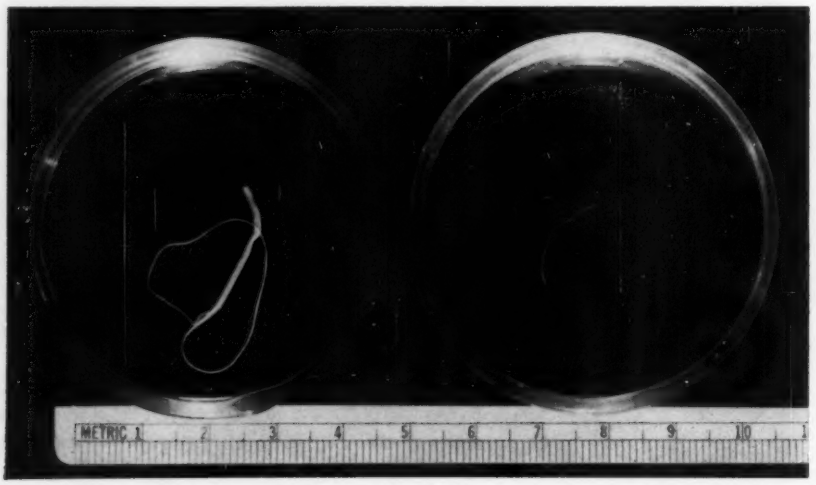


FIGURE 4. Individuals of *Hymenolepis diminuta* grown in vitro for 15 days (left) and for 7 days (right).

aggregate of many materials. Some of these have been found to be essential. Serum was tried in place of whole blood plasma without success. In like manner, no active growth occurred when either the chick embryo extract or yeast extract was omitted, or added before the blood plasma and Tyrode's had gelled. Methyl testosterone was added as a result of the studies of Beck (1952) on gonadectomies and gonadal hormones. Without methyl testosterone, some growth occurred, but differentiation of the young proglottids was inhibited.

A more effective method of excysting larvae (Rothman, 1959) must be used, since the method used so far requires large numbers of cysticercoids of which only a low percentage excysts. Furthermore, the time required for excystment is variable.

Another problem is that, to date, single individuals have not been successfully grown in vitro. Of an initial 20 or so larvae per tube, 2 and 4, and occasionally 6 adult worms remain in a final culture. No growth occurred when tubes were innoculated with single larvae. No traces of the disappearing larvae were ever found in either the tubes or collecting bottles. It has been suggested that some of these worms in the early stages autolyse and supply some essential substance to those worms which grow to maturity. However, this is not likely, except perhaps in the very early growth period, since the medium is being continually exchanged.

At present the culture unit is being redesigned to eliminate as many of the manipulatory problems as possible keeping, however, the primary principles intact. In vitro cultures of other species are in progress and have already produced some positive results. Studies to evaluate the necessity of each component of the medium and its relation to growth are being carried out. It is hoped that this method, even though it is only the first step to a new tool for the parasitologist, will be applicable to other organisms which up to now have been refractory to in vitro culture.

SUMMARY

A method is described which employs a continuous flow of medium in the in vitro cultivation of the cyclophyllidean cestode *Hymenolepis diminuta*.

Cysticercoids dissected from beetles were excysted and cultured to adults with proglottids containing developing oncospheres. These results were reproducible. Total length changes of the growing worms were recorded on different days of culture.

Problems associated with this method are discussed.

REFERENCES

BECK, W. J. 1952 Effect of gonadectomy and gonadal hormones on singly established *Hymenolepis diminuta* in rats. Exp. Parasit. **1**: 109–117.

BERNTZEN, A. K. 1960 An effective method for the in vitro culture of *Hymenolepis diminuta*. J. Parasit. **46** (supp.): 147.

MUELLER, J. F. 1958 In vitro cultivation of the sparganum of *Spirometra mansonoides* to the infective stage. J. Parasit. **44** (supp.): 14–15.

MUELLER, J. F. 1959 The laboratory propagation of *Spirometra mansonoides* (Mueller, 1935) as an experimental tool. III. In vitro cultivation of the plerocercoid larva in a cell-free medium. J. Parasit. **45**: 561–569.

PARKER, R. C. 1950 Methods of Tissue Culture. Harper and Brothers, New York. 2d Ed., revised.

ROTHMAN, A. H. 1959 Studies on the excystment of tapeworms. Exp. Parasit. **8**: 336–364.

SCHILLER, E. L., READ, C. P. and ROTHMAN, A. H. 1959 Preliminary experiments on the growth of a cyclophyllidean cestode in vitro. J. Parasit. **45** (supp.): 29.

SMYTH, J. D. 1955 Problems relating to the in vitro cultivation of pseudophyllidean cestodes from egg to adult. Ibér. Parasit. Tomo Extraordinario por Prof. Lopez-Neyra, pp. 65–86.

SMYTH, J. D. 1956 Studies on tapeworm physiology. VIII. Occurrence of somatic mitosis in *Diphyllobothrium* spp. and its use as a criterion for assessing growth in vitro. Exp. Parasit. **5**: 260–270.

SMYTH, J. D. 1958 Cultivation and development of larval cestode fragments in vitro. Nature **181**: 1119–1122.

TAHMISIAN, T. N. 1945 An oxidation reduction method for stain differentiation. Stain Tech. **20**: 11–12.

RESEARCH NOTE

SOME PARASITES OF MAN IN NYASALAND.

During a 9-month period, from July 1959 to April 1960, a total of 458 persons were examined for parasites as part of a drug evaluation project. Those examined were lepers and/or members of their families from the leprosarium located near the hospital grounds of the Malamulo Hospital at Makwasa (near Blantyre) Nyasaland. Others studied were students of the training school, hospital workers, and hospital patients.

Soft or liquid stools were examined by the warm saline preparation and with iodine. Formed stools were also examined by this procedure, but most frequently by the formalin-ether concentration method, except that approximately 10 percent were concentrated by the zinc sulfate method. Urines were concentrated by sedimentation at first, and later by centrifugation.

Of a total of 458 stool and urine specimens examined from as many persons, 337 (73.6 percent) were positive for one or more parasites. There were 144 single infections, in which only one species of parasite was found, 102 double infections, 57 triple, 17 having four species of parasites, 10 with five species, 6 with six species, and 1 with seven.

Ninety-six (21 percent) persons had *E. histolytica*. All of them exhibited the cyst form and nine the trophic stages. No measurements were made, but on the basis of comparative sizes, all *E. histolytica* positive stools had the large race, and three of these also the small race.

Amebiasis in this part of Africa was found to be of the chronic, asymptomatic cyst-carrier type.

There were 117 stools positive for *E. coli* (25.5 percent). These had the cyst stage only present, except that one had the trophic form only, and three had both. The smaller amebas are not considered as representing their actual incidence because only the cyst stages were recorded. On this basis *Iodamoeba bütschlii* was seen in 34 persons (7.4 percent) and *Endolimax nana* in 20 (4.4 percent).

Of the helminth infections, hookworm ova were most frequently seen. These were present in nearly half (45.2 percent) the persons examined. Table I lists the number of infected persons and percent of parasite infections observed.

TABLE I. *Parasite incidence in 458 fecal specimens from as many persons examined in Nyasaland.*

Parasite	Positive specimens	
	Number	Percent
Entamoeba histolytica	96	21.0
Entamoeba coli	117	25.5
Chilomastix mesnili	5	1.1
Giardia lamblia	2	0.4
Hookworm	207	45.2
Schistosoma haematobium	96	21.0
Schistosoma mansoni	43	9.4
Ascaris lumbricoides	39	8.5
Strongyloides stercoralis	26	5.7
Trichuris trichiura	7	1.5
Taenia sp.*	1	0.2
Hymenolepis nana	1	0.2

* Examination method used rarely reveals this infection.

The authors are grateful to Parke, Davis and Co., for support of the project making this work possible.—EDWARD D. WAGNER AND HOWARD S. BURNETT, *Department of Microbiology, School of Medicine, College of Medical Evangelists, Loma Linda, California.*

A MECHANICAL APPARATUS FOR SCREENING WORM EGGS FROM FECES

CHARLES H. HILL AND RALPH E. ZIMMERMAN

Animal Disease and Parasite Research Division, Agricultural Research Service,
U. S. Department of Agriculture, Beltsville, Maryland

Previous investigations carried out by the senior author on the swine whipworm, *Trichuris suis*, required large numbers of clean worm eggs. These eggs could be obtained by manual washing of feces through a series of wire screens, but the task was time-consuming and tedious. Therefore, a mechanical device was designed that has been used successfully in separating nematode eggs from the feces of swine, sheep, and dogs as previously described by the authors (1954, 1957). This report gives instructions for constructing and operating such a machine, including results of critical tests. The modifications did not destroy the usefulness of the machine as a mixer.

General Description of the Apparatus

Three basic considerations governed the design of the apparatus:

1. The suspension of raw feces containing parasite eggs in water within a screened container; 2. the continuous cleaning of the cylindrical surface of the screen by a revolving paddle; and 3. the washing and flushing-out of the fecal contents by means of a stream of water directed inside the screened container from a piped supply from a water tap.

The apparatus (figs. 1 and 3) has four components: (1) a planetary-type electric mixing machine which is (a) the source of mechanical power for the paddle which rotates rapidly as it revolves in a circular path close to the surface of the cylindrical screen and (b) a base for (2) a copper bucket which was designed to serve as an intermediate catch basin and to receive and support (3) a screened, cylindrical basket with connections for supplying running water, and (4) a paddle or agitator which is attached to the revolving head of the mixer. The materials required for making these components are listed at the end of the paper and are specifically referred to in the appropriate section.

Received for publication October 6, 1960.

Planetary-type electric mixer. This mixer is the model K5-A made by the Hobart Manufacturing Company of Troy, Ohio. The mixing blades furnished by the manufacturer are not used.

The copper bucket. This component (figs. 1 and 3) is constructed of 20-gauge copper; it is 7.5 inches high and has an inside diameter of 8 inches. It should be made by a professional sheet metal worker, who should be careful to make the bottom flat and the sides perpendicular to it. The bucket is attached to the U-shaped bracket of the mixing machine by two perforated metal ears of 1 inch by ⅛ inch brass stock that are soldered to its sides so that the bucket is held firmly in a horizontal position. The upper arms of the "ears" serve as handles for lifting the bucket manually. Since the bottom of the bucket will be above the concave base of the mixing machine, copper shims are soldred to the bucket in such a way as to prevent it from moving or rocking, while the machine is in operation. A strip of 20-gauge copper, ⅜ inch wide, is soldered along the upper edge of the bucket to strengthen it and to serve as a base for brackets to which the arms of the basket are attached. A ¾-inch hole is punched near the base of the bucket on the side that will be nearest the operator and a brass ¾-inch gate valve is inserted and soldered in place. A short length of ¾-inch I.D. copper pipe is then soldered to the gate valve to facilitate the attachment of plastic tubing. (The pipe connection seen at upper right of the bucket in figures 1, 3, and 4 is of no value.)

The basket frame. The basket frame, (fig. 2) is made of four rings (top ring, ½ inch wide, two middle rings, ¼ inch wide, and one bottom ring, 1 inch wide) that are accurately sawed from brass tubing 4-inches O.D. and ⅛ inch thick, and are held together by four pieces of ¼- by ⅛-inch brass stock which form the legs and arms of the basket. Pieces of this stock,

10¾ inches long, are bent at right angles 7¾ inches from one end, so that the short arm of each piece will reach from the top ring to the outer margin of the bucket. These pieces are soldered to the rings 90° apart on their circumference as shown. The top ring is located ¼ inch below the upper surface of each arm and the bottom ring is soldered onto the legs so that its lower surface is exactly seven inches from the upper surface of the top ring. The middle rings are then soldered equidistant from each other and from the top and bottom rings. Care should be exercised to avoid applying excess heat at any one point since adjacent finished joints might be melted and pieces should be aligned in such a way as to make as nearly a perfect cylinder as is possible.

The revolving paddle. The construction of the paddle or agitator (fig. 1) requires the services of a machinist to bore and mill its adaptor head so that it will fit the planetary-head pin of the mixing machine. The head of any of the mixing blades provided by the manufacturer of the machine may be used as a model. The head is made from a solid brass rod, measuring 1¼ by 1½ inches, which is machined to fit the planetary-head pin. A hole is then drilled in the center of the lower flat surface into which is soldered a piece of ¾-inch O.D. brass pipe, 6¾ inches long, having four longitudinal slits, sawed or milled on the outer surface, equidistant from each other and wide enough to take 1/16 inch brass stock. Four brass vanes, ⅜ by 6½ inches, of 1/16-inch brass stock are then soldered in the slots as shown. The pipe is closed by soldering a copper cap over the open end.

Fitting the basket frame. Each basket frame must be hand-fitted to the bucket so that it is accurately centered under the revolving planetary head of the mixer and held firmly with its sides parallel to the blades of the paddle. First, file the legs so that when the frame is placed on the floor of the bucket, the arms almost touch its rim. Then, with the agitator attached to the planetary-head pin, and the basket frame held by hand and approximately centered thereunder, operate the machine at the slowest speed. It will be found that the paddle blades will clear the rings of the basket frame by ⅛ to 1/16 inch. By filing or grinding the ends of the legs, any lack of fit between the revolving paddle and the frame can be corrected. The plumbness of frame and paddle should be established with the arms of the basket always in the same position relative to the circumference of the bucket. After the frame is accurately centered under the planetary head and its position checked by operating the ma-

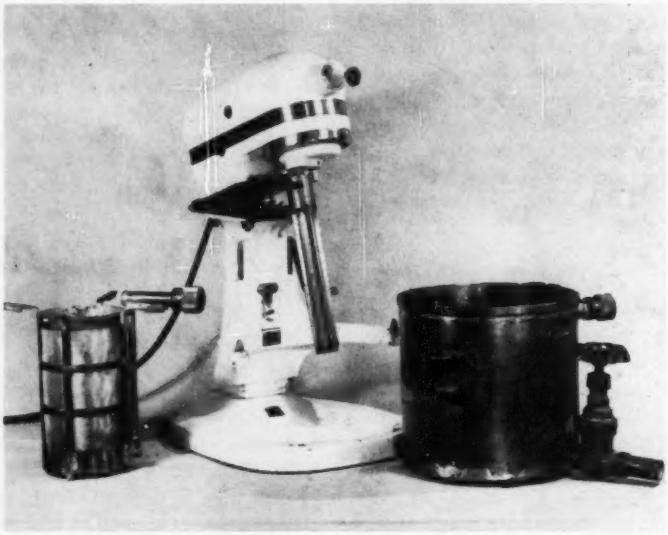


FIGURE 1. *Planetary mixing machine with hinge and paddle attached, screened basket and copper bucket as described in the text.*

chine slowly with agitator in place, the points where the arms meet the edge of the bucket are marked and stops of ¼- by ⅛-inch brass stock are soldered to each arm at this mark. To secure the basket frame further to the bucket, an inverted U-shaped collar made from this stock is soldered to the left rear side of the rim of the bucket (figs. 1 and 3) at the exact spot where the appropriate arm of the frame rests and into which it will fit. An open, U-shaped slot, made with the same material, is provided on the rim opposite this position into which the opposite arm of the frame will rest. The apparatus can now be tested by running at a fast speed, after which further adjustments can be made as previously described.

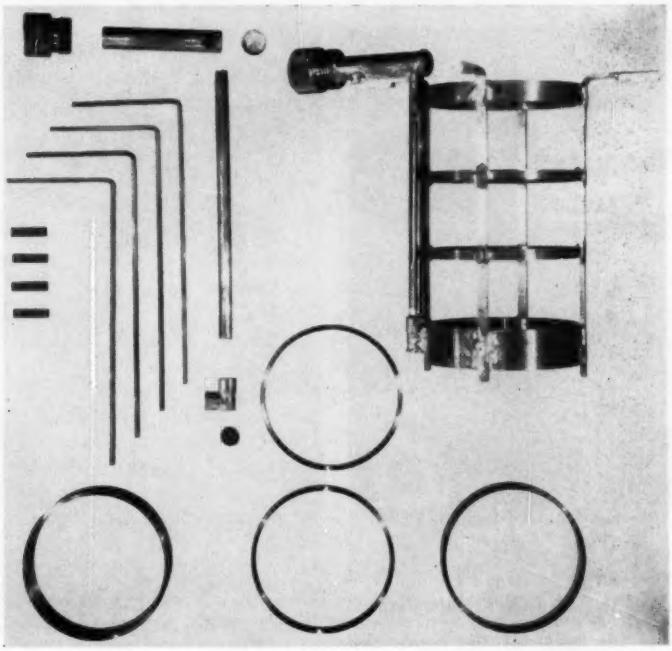


FIGURE 2. Assembled basket frame and parts from which it was made.

Installing pipe on basket frame. Drill a ⅜-inch hole, ⅜ inch from one end of the 3-inch length of ½-inch copper pipe (fig. 2). Make eight longitudinal slits, ¼ inch deep in one end of the ¼-inch copper pipe and insert the slit end into the hole; bend the eight tabs at right angles and solder. A standard, ½-inch wrought copper cap is sawed through parallel to the flat surface to make a disk approximately ⅛ inch thick. This shallow cap is soldered to the end of the ½-inch pipe nearest the joint. A hole to take the ¼-inch tee is drilled in the bottom ring of the basket frame adjacent to the right front leg and the tee is inserted to a snug fit. The ½-inch arm of the ell is held close and approximately parallel to the front right arm of the basket frame and the open end of the ¼-inch pipe directed into the tee and soldered without disturbing the alignment. The tee and the ½-inch pipe may then be soldered to the basket frame as shown. The bottom arm of the tee is now sawed flush and closed by soldering over it a small disc of copper. The projecting portion of the tee on the inside of the basket frame is filed flush with the ring. A "Quick-Fit" garden hose connector is then soldered to the free end of the ½-inch arm of the ell.

Selecting screens for the basket. Screens having 200 meshes to the inch and openings of 74 microns were satisfactory for separating most of the common worm eggs from feces. This mesh will also allow the processing of raw feces without preliminary treatment to remove the coarser particles and will not wear out as rapidly or clog up as readily as finer and more

delicate screens. Wire cloth having meshes of 80, 100, 200, and 400 meshes to the inch and openings ranging from 170 to 38 microns, respectively, have been used by the senior author in this apparatus.

Cutting and attaching wire cloth to basket frame. A rectangle 12 by 6.5 inches and a circle 4 inches in diameter are cut from wire cloth of the selected mesh. The surface of the rings of the basket frame to which the cloth is to be attached is tinned with solder; the rectangle is fitted snugly to the inside of the frame and clamped to the top and bottom rings with small laboratory clamps. Begin soldering at one corner of the rectangle and proceed along the top ring and wire cloth edge toward the adjacent corner. Remove clamps as the soldering progresses. When the opposite corner is reached, the cloth is lapped and soldered. This process is repeated on the bottom ring. The lapped vertical edges are now soldered through the open ends of the basket. The screen over the outlet of the tee is pierced and is soldered around the opening. The circular piece of wire cloth is then soldered tightly to the lower surface of the bottom ring.

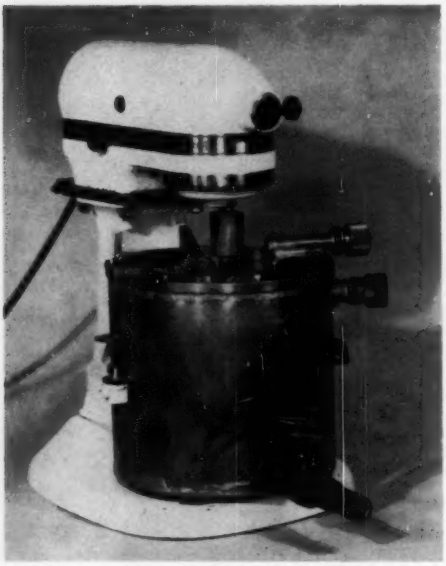


FIGURE 3. Closeup view of assembled apparatus.

Operation. After setting up the apparatus (fig. 4) and removing the motor housing and paddle, add raw feces to the basket, fitted with the 200-mesh screen, until it is slightly more than half full. This will require about 500 cc of hog or cattle feces or about 300 g of the drier sheep pellets. Larger quantities of feces may be processed, but the time required will be prolonged out of proportion to the amount of the increase. Water should be added until it is within an inch of the top of the basket and 2 inches deep in the bucket. The motor housing and paddle are replaced and the machine started and operated at slow speed. As the level of the fecal suspension drops, water can be added slowly and continuously from the tap at a rate that will not cause the basket to overflow. The gate valve is partially opened and the suspension is allowed to drain into the large glass jar. As the gelatinous material and fine particles in the sample decrease, the flow of water can be accelerated, the outlet valve opened wider, and the speed of the motor increased. The volume of fluid draining through this valve is not critical but it should be adjusted during the initial processing so that water covers the lower half of the basket. When water is passing through the screen freely, this valve can be opened wide. Toward the end of the operation, the water can be turned on full force and the basket flushed out without danger of its over-

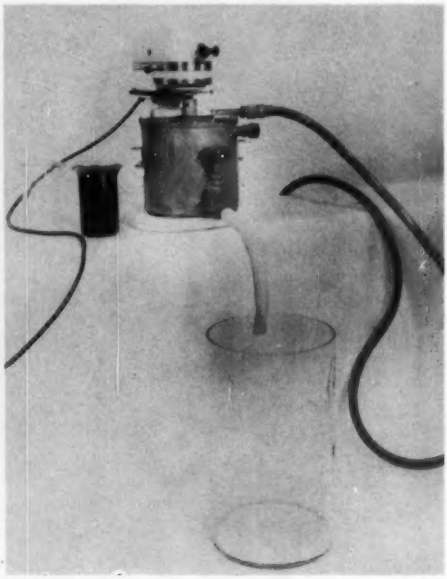


FIGURE 4. Apparatus ready for operation.

flowing because of a clogged screen. The revolving paddle should not be allowed to operate in very heavy feces or against fibrous debris remaining in the bottom of the basket without water being present. The proper regulation of the machine depends upon the experience and judgment of the operator. Raw feces should be passed through 80- or 100-mesh screens before being passed through those finer than 200-mesh.

The suspended material in the jar is allowed to sediment for about one hour and the supernatant is then siphoned off. Additional sedimentations can then be made in the same or smaller jars. The eggs collected by this process may be further concentrated, and cleaned by using flotation techniques combined with centrifugation. They can then be counted by the usual dilution methods. The 14-liter jar used to receive the suspension of worm eggs and fine debris was sufficient in practically all instances to permit the thorough washing of the 300- to 500-gram samples of feces on which the tests were run.

To facilitate the removal and filling of the basket, small "wings" can be soldered to the heads of the bolts supplied with the machine. These bolts fasten the motor housing to the stand and can be loosened manually to allow the removal of the motor housing and paddle without disturbing the remainder of the apparatus. The original bolts can also be replaced by winged bolts obtained especially for this purpose. Access to the basket can also be made easier by fitting a heavy metal hinge to the mixer as shown in figures 1 and 3. Since this hinge will differ with the model of the machine for which it is made, and because it is not absolutely essential for the proper functioning of the apparatus, details of its construction have been omitted.

Tests with the Apparatus

The consistency of hog and calf feces permitted accurate measurement of volume, whereas the relatively dry pellets of sheep feces had to be crushed and mixed with water in order to produce a mixture on which comparable volumetric measurements could be made. For the tests with *Trichuris* eggs, from 2,500 to 3,000 cc of undiluted swine feces were mixed for 20 minutes in the mixing bowl of a similar but unmodified mixing machine. In the other tests, smaller amounts of feces were similarly mixed but for slightly shorter periods of time. The number of eggs per cubic centimeter of the original sample was estimated by standard egg-counting technique. Quantities of 500 cc were then removed from the mixing bowl and were screened in the machine. After sedimentation, the concentrated eggs and fine fecal material were re-suspended, the volume adjusted to 500 cc and the number of eggs was again estimated. The residues from the screen after washing were also examined microscopically for worm eggs after washing had been completed.

Results: The results of these experiments are recorded in table 1. These data show that the efficiency of the apparatus under the experimental conditions was very high. No worm eggs were seen during microscopic examination of residues remaining in the basket. Although critical tests were not made, oocysts of *Eimeria debliecki* were successfully removed from pig feces by screening the specimen through a 400-mesh screen.

TABLE I. *Average number of worm eggs present in swine, sheep, and cattle feces and average percentages recovered by screening through the described apparatus.*

Source	Parasite	Prescreening		Post screening		Percent Recovered
		Runs made (No.)	E.P.G. (Av.)	Runs made (No.)	E.P.G. (Av.)	
Hog	*Trichuris*	7	11,257	14	11,243	99.9
	Ascaris	2	20,900	4	20,850	99.8
	Metastrongylus	4	2,200	10	2,480	112.0*
	Oesophagostomum	3	1,150	4	1,300	113.7*
Sheep	*Trichostrongylus*	2	1,800	4	1,750	97.2
	Haemonchus	1	1,000	1	1,000	100.0
	Ostertagia	2	1,200	2	1,200	100.0
Calf	*Cooperia*	2	400	2	400	100.0

* Percentages more than 100 are attributed to experimental error.

SUMMARY

The materials and procedures for converting an electrical, planetary mixing machine into an apparatus for separating worm eggs from feces are described. Directions for operating the apparatus are also given.

Critical tests on 300-g samples of sheep feces and 500-cc samples of swine and cattle feces, respectively, demonstrated that more than 99 percent of the worm eggs contained therein were removed in approximately 5 minutes running time when a 200-mesh screen was used. Evidence was obtained showing that coccidial oocysts could be separated from swine feces by using a 400-mesh screen.

The changes made in the machine did not destroy its usefulness as a mixer.

Literature Cited

Hill, C. H. and Zimmerman, R. E. 1954 Use of a standard planetary-type mixing machine for separating eggs of the swine whipworm, *Trichuris suis*, from feces. J. Parasit. 40 (supp.): 32.

——— and ———. 1957 Further uses of a planetary-type mixing machine adapted for separating worm eggs from feces. J. Parasit. 43 (supp.): 31.

List of Materials

There follows a list of all materials and parts needed to modify a Model K5-A Hobart Planetary Mixing Machine for screening eggs from feces and necessary accessories to make the apparatus operational. The quantities and dimensions of the materials listed are applicable to this model only.

Cost of items and labor are omitted because many of the parts may be available in institutions with mechanical shops, and labor charges may not apply in other situations. The cost of the parts for converting the mixer at this laboratory was approximately $50.00.

A. Copper bucket
   1. Copper sheet, 36-oz., 20-gauge, 9 in. × 40 in.
   2. Brass stock, 1 in. × ⅛ in. × 7⅛ in.

B. Cylindrical basket
   1. Brass tubing, 4 in. (OD) × ⅛ in × 3 in.
   2. Brass stock, ¼ in. × ⅛ in. × 48 in.
   3. Wire cloth, stainless steel, 200-mesh, 12 in. × 12 in.

C. Paddle or agitator
   1. Solid brass rod, 1¼ in. × 1½ in.
   2. Brass pipe, ¾ in. (OD) × 6¾ in.
   3. Brass stock, ⅜ in. × 1/16 in. × 26 in.

D. Plumbing stock
   1. Copper water pipe:
      a. ¾ in. (ID) × 2 in.
      b. ½ in. (ID) × 3 in.
      c. ¼ in. (ID) × 6⅝ in.
   2. Wrought copper cap, ½ in.
   3. Wrought copper tee, ¼ in.
   4. Brass gate valve, ¾ in.
   5. "Quick-Fit" adapter for ¾ in. (OD) plastic hose.

E. Miscellaneous
   1. 2 wing bolts ¼ in. in diameter, or 1 hinge of 3/16 in. metal; 5⅝ in. long by 4 in. wide, and screws to fasten it to the mixer.
   2. Plastic garden hose, 10 ft. ¾ in. OD, ½ in. ID.

A SIMPLE PROCEDURE FOR RECOVERING SCHISTOSOME EGGS IN MASS FROM TISSUES*

LAWRENCE S. RITCHIE AND LUIS A. BERRIOS-DURAN
U. S. Army Tropical Research Medical Laboratory, Fort Brooke, Puerto Rico

The need for obtaining schistosome eggs in mass for pathologic and immunologic studies has been stated by Smithers (1960) and Coker and Lichtenberg (1956). These investigators described techniques for egg recovery; the former utilized tissue digestion and subsequent washing by sedimentation, while the latter separated eggs from tissue homogenates by differential sedimentation, including the use of a sugar solution.

In an attempt to obtain clean schistosome eggs from tissue homogenates by continuous-flow washing, we noted a layering tendency on the part of the washing solution and the homogenate, both of which were of 1.7 percent saline to suppress hatching (Rodriguez-Molina, et al, 1958). When the concentration of the washing medium was increased to 2 percent, the layering occurred quite consistently, and the phenomenon was successfully and simply applied to mass recovery of *Schistosoma mansoni* eggs, as they readily sedimented through the interface into the clear solution.

EQUIPMENT

The following items constitute the equipment and materials utilized in the egg-recovery process: Waring blendor; saline, 1.7 percent; detergent (Triton—100); parasitologic sieves (5″), Nos. 40 to 160 (five); glass and rubber tubing; screw clamp; carboy, 5-gal; saline, 2.0 percent; beakers, 2000 and 1000 ml; side-arm Erlenmeyer flask; Erlenmeyer flasks (small); aerating stone; suction apparatus.

THE PROCEDURE

In order to obtain livers or intestines heavily loaded with eggs, mice or hamsters are exposed to 200 and 500 cercariae, respectively, and sacrificed after about 7 weeks. Three organs are homogenized at a time by Waring blendor with 500 ml of 1.7 percent sodium chloride solution for 1 minute. Triton 100 (8 to 10 drops) is added, preferably near the end of the homogenization, as this reduces the foam. If intestines are to be used, they should be washed free of feces; regardless, they are not so successfully handled as livers. The homogenate is poured thru a series of parasitologic sieves, collected with rinsings (a total of about 700 ml.) in a 2000 ml beaker, and transferred to a sidearm Erlenmeyer flask.

The next and critical step is the introduction of 2 percent salt solution at the bottom of the sidearm flask. A relatively heavy aeration stone is fitted to a foot-length of plastic or rubber tubing which in turn is adapted to a carboy reservoir by rubber tubing. The rate of flow is controlled at 70 to 100 ml per minute by means of either a screw clamp, or metal valve which is used for aquarium aeration lines. A sharp layering between the homogenate and the 2 percent sodium chloride solution should become evident shortly. The flow is continued until the homogenate is all discarded thru the sidearm overflow.

After sedimentation is complete, the contents of the sidearm flask are reduced to about 200 ml or less by means of a suction device. The volume of solution is further reduced by sedimentation in a series of progressively smaller Erlenmeyer flasks until the volume is about 10 ml. Further handling of the eggs depends upon their use. If they are to be lyophilized, the 2 percent saline must be replaced with distilled water. In making this transfer, hatching must be suppressed, which is accomplished by using refrigerated distilled water (4 C) in three washings with centrifugation.

Received for publication January 19, 1961.

* This material has been reviewed by the Office of The Surgeon General, Department of the Army, and there is no objection to its presentation and/or publication. This review does not imply any indorsement of the opinions advanced or any recommendation of such products as may be named.

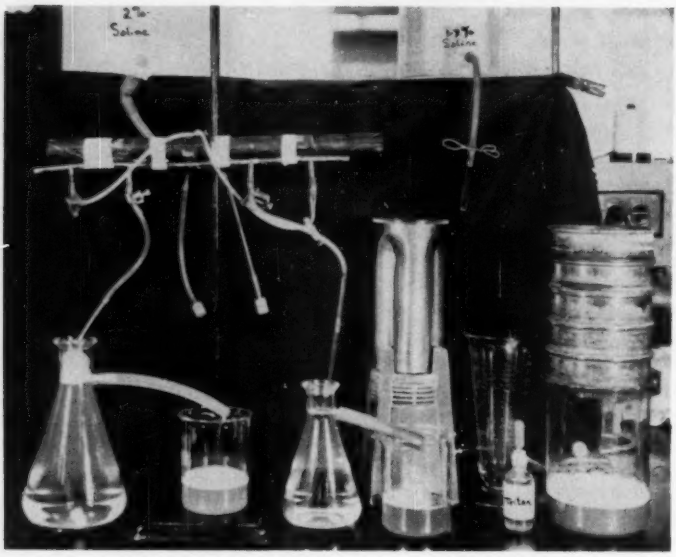


FIGURE 1. Equipment and materials for mass recovery of schistosome eggs from tissues.

DISCUSSION

It is expected that individuals using this procedure will find desirable modifications, because each step has not been varied experimentally.

The advantage of using more than three livers or intestines in one operation is apparent, but thus far, layering has been less effective with increased numbers of organs and proportionate amounts of saline. The possibility of incorporating this advantage, however, has not been exhausted. To compensate, several sidearm flasks for layering are set up by means of an aquarium aeration line with multiple valves and spigots (fig. 1). The use of 1.7 percent sodium chloride serves to suppress hatching of the eggs (Rodriguez-Molina et al, 1958), and the detergent is critical as it tends to enhance the layering; undoubtedly other than triton will serve as well. The number of sieves can probably be varied from the number reported. In fact they have been excluded with fair results, but a repeat washing by layering was necessary to obtain clean eggs. The choice of 2 percent sodium chloride solution for the layering has been evaluated and found to be optimal. The use of the aquarium stone is deemed critical, as it disperses the current of flow, thus lessening disturbance to the layering action. It also acts as a weight for the end of the tube which introduces the 2 percent saline at the bottom of the flask. For most effective layering, it is well to continue the flow until the homogenate completely overflows. Completion of egg sedimentation can be determined by the unaided eye or by hand lens. If layering is imperfect the first adjustment that should be considered is increased dilution of the homogenate.

We believe the method described is simpler than those of Smithers (1960) and Coker and Lichtenberg (1956), primarily because of the tedious demands for sedimentation required in their techniques and the additional tissue digestion included in the method of Smithers. There appears to be little difference in the quality of the eggs obtained by our method as compared with theirs. The differential sedimentation of mature from other types of eggs does not appear to be critical, because at 7 weeks dead eggs are rarely present. According to our judgment they are either mature or immature. The eggs obtained by our recovery method are quite acceptable for the circumoval test of Oliver-Gonzalez (1954), for antigen preparation, and other needs.

We have found that the layering phenom-

enon can be used for the recovery of worms from bloody perfusate, particularly the schistosomula in the early stages of infection, 2 to 4 weeks after exposure. After observing our procedure, Rowan and Gram (1959) adapted the layering phenomenon to the recovery of eggs from large volumes of sewage. They also reported obtaining eggs from feces, but their apparatus was complicated and of limited use. We believe our method may be adaptable for recovery of eggs from feces when a proper dilution factor, and possibly improved straining, have been incorporated. Straining of feces with suction through fine-mesh cloth in a Büchner funnel has been successfully tried.

SUMMARY

A procedure has been developed which the authors believe is more convenient for recovery of schistosome eggs from tissues in quantities sufficient for serologic, immunologic, or other types of investigation requiring eggs. The primary feature is linked with the fact that a sharp layering can be obtained between tissue homogenates (in 1.7 percent NaCl) and a 2.0 percent saline solution. Layering is accomplished by introducing the clear saline through an aerating stone at the bottom of a flask containing the homogenate. As the latter is lifted the eggs fall through the interface into the clear solution.

REFERENCES

COKER, C. M. AND LICHTENBERG, F. 1956 A revised method for isolation of *Schistosoma mansoni* eggs for biological experimentation. Proc. Soc. Exp. Biol. Med. **92**: 780–782.

OLIVER-GONZALEZ, J. 1954 Anti-egg precipitins in the serum of humans infected with *Schistosoma mansoni*. J. Inf. Dis. **95**: 86–91.

RODRIGUEZ-MOLINA, R., OLIVER-GONZALEZ, J. AND SERRANO, D. G. 1958 Studies on immunity to bilharziasis mansoni: evaluation of the circumoval precipitin test. Bull. World Hlth. Org. **18**: 1105–1107.

ROWAN, W. B. AND GRAM, A. L. 1959 Quantitative recovery of helminth eggs from relatively large samples of feces and sewage. J. Parasit. **45**: 615–621.

SMITHERS, S. R. 1960 The isolation of viable schistosome eggs by a digestion technique. J. Roy. Soc. Trop. Med. Hyg. **54**: 58–70.

ANNOUNCEMENT

by

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Bureau of State Services
Communicable Disease Center
Laboratory Branch
Atlanta 22, Georgia

SCHEDULE OF LABORATORY REFRESHER TRAINING COURSES
(July 1961–June 1962)

DATES	COURSES	DURATION
Sept. 11–Oct. 6	Laboratory methods in medical parasitology. Part 1. Intestinal Parasites (800). Closing date: July 31, 1961.	4 wks
Sept. 18–29	Fundamentals of virology (819). Closing Date. August 7, 1961.	2 wks
Oct. 2–13	Fluorescent antibody techniques in streptococcus. Grouping (860). Closing date: August 21, 1961.	2 wks
Oct. 9–27	Laboratory methods in medical parasitology. Part 2. Blood Parasites (801). Closing date: August 28, 1961.	3 wks
Oct. 23–Nov. 3	Fluorescent antibody techniques in the Public Health Laboratory (845). Closing date: September 11, 1961.	2 wks
Oct. 30–Nov. 17	Laboratory methods in the diagnosis of viral and rickettsial diseases (820). Closing date: September 18, 1961.	3 wks
Nov. 27–Dec. 1	Laboratory methods in the diagnosis of rabies (826). Closing date: October 16, 1961.	1 wk
Dec. 4–8	Bacteriophage typing of staphylococci (856). Closing date: October 23, 1961.	1 wk
Jan. 8–Feb. 2	Laboratory methods in medical mycology (815). Closing date: November 27, 1961.	4 wks
Jan. 15–26	Laboratory methods in the diagnosis of tuberculosis (855). Closing date: December 4, 1961.	2 wks

(*Continued on page 468*)

A PERFUSION PROCEDURE (PERF-O-SUCTION) FOR RECOVERY OF SCHISTOSOME WORMS

MYRON G. RADKE*, L. A. BERRIOS-DURAN AND K. MORAN**
U. S. Army Tropical Research Medical Laboratory, APO 851, New York, New York

The scarcity of adult schistosome worms has limited physiologic and immunologic investigations of schistosomiasis. Perfusion techniques have been described (Faust and Meleney, 1924; Yolles et al, 1947, and Pan and Hunter, 1951) which facilitated the collection of worms from infected animals. The principal objection to these procedures is the small number of animals a technician is able to perfuse per day.

A relatively simple and rapid method for collecting large numbers of schistosomes from the portal circulation of infected animals was devised and has been used successfully for several years at the U. S. Army Tropical Research Medical Laboratory. This technique was briefly described (Radke et al, 1957) in abstract form.

MATERIALS AND METHODS

Description and use of equipment. Three pieces of equipment were used in the "Perf-O-Suction" technique: (1) automatic pipetting machine*** with foot switch, (2) suction apparatus, and (3) special autopsy boards (see figure 1). The pipetting machine which provided physiologic saline (3 ml per stroke, 21 times per minute) under pressure was equipped with a 40-inch rubber tube attached to the machine's syringe and the free end was fitted with a hypodermic needle adaptor. The suction apparatus consisted of a separatory funnel (500 or 1000 ml) containing 200 ml 0.85 percent saline. The funnel was fitted with a two-hole rubber stopper through which two pieces of glass tubing had been inserted. One of these was connected by rubber tubing to a vacuum source; the other piece of glass tubing was inserted at least 0.25 inches below the saline in the funnel and the free end was fitted with rubber tubing of sufficient length to reach the autopsy board so that the perfusate could be readily aspirated. By adjusting

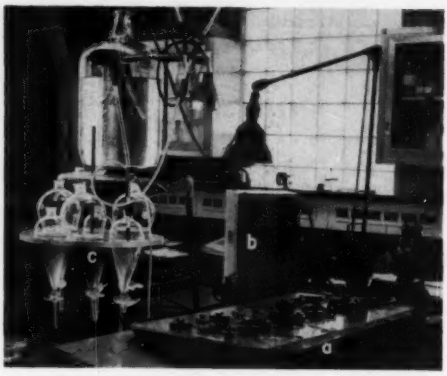


FIGURE 1. A practical arrangement of the "Perf-O-Suction" equipment. a, small animal autopsy board; b, automatic pipetting machine with foot switch; c, adjustable rotating stand with suction apparatus.

the negative pressure of the vacuum line, it was possible to regulate the amount of suction for retrieving the perfusate (blood, saline, and worms). The air and perfusate entering the separatory funnel through the intake tube provided sufficient agitation to prevent the schistosomes from becoming entangled in congealing blood. An erlenmeyer flask was attached to the vacuum line to trap worms in the event the separatory funnel overflowed.

The perfusion boards were constructed from 0.25-inch plastic (16 × 30 inches). Animals to be perfused were clamped by the feet with four clothespins to Autopsy boards (fig. 2). For mice, 2-inch clothespins† were fastened to the board so that the rectangle formed from the fulcrums of the clothespins measured 3⅛ inches by 5⅝ inches. Hamsters were fastened with three-inch clothespins†† which were secured to the board forming a 6.5-inch square. Mice were prepared and perfused in groups of five, and hamsters in groups of three. Perforations on the boards, located on both sides of the positioned animals, permitted the collection and examination for worms of any excess fluid. Duplicate autopsy boards were used to expedite worm recoveries; while one group of animals was perfused, another was prepared.

† Mastro Plastics Corporation, 3040 Webster Avenue, New York 67, New York

†† Nalle Plastics, Inc., Austin, Texas

Received for publication October 10, 1960.

* Present address: Division of Communicable Disease, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington 12, D. C.

** Present address: Walter Reed Army Medical Unit, Fort Detrick, Maryland.

*** Brewer Automatic Model 40, Baltimore Biological Company, Baltimore, Maryland

FIGURE 2. Technique used to flush schistosomes from the liver. Needle was locked into place by inserting it between the ribs through the diaphragm into the hepatic sinus. A natural pocket occurring at the point of the severed hepatic portal vein (arrow) provided a site for retrieving worms.

Animal preparation and perfusion. Chloroform-killed animals are clamped in a supine position on the autopsy boards where the animals remain until perfused. An incision from the sternal apex to the pubic region and transverse incisions at the sternum are made to expose the abdominal cavity. Animal preparation is completed by removing the diaphragm and one side of the thoracic cage. A 1.5 inch 23-gauge hypodermic needle, attached to the tubing from the pipetting machine, is inserted into the thoracic aorta. The hepatic portal vein is severed and pressurized saline from the automatic pipetting machine is injected to dislodge the worms from both the liver and mesenteric veins. At first, the machine must be operated briefly to prevent an excessive surge of blood, saline, and worms which cannot be retrieved adequately by the suction apparatus. Following this initial control of perfusate, the machine can be operated for longer intervals of time. No blood vessels are ligated in small animals except when separate worm counts are desired from the liver and mesenteric veins, and this can be accomplished by blocking the hepatic portal vein with a small hemostat. For separate organ counts, it is recommended that the liver be cleared of worms first by inserting the needle into the hepatic sinus (fig. 2), and then the mesenteric veins are cleared as described previously. In larger animals, such as rabbits and monkeys, the organs must be perfused separately.

Evaluation of the "Perf-O-Suction" technique. Since the albino mouse and the golden hamster are used as experimental hosts in many investigations on *Schistosoma mansoni,* our evaluation of the technique's efficiency was limited to these animals. All animals were perfused individually and the worms harvested from the liver and mesenteric veins were pooled. These organs were then pressed between two plastic sheets and examined under the dissecting scope (low power) for remaining schistosomes. No additional worms were found in 83.9 percent of 587 perfused mice. The technique made possible the recovery of 98 percent of the 14,956 worms present in these mice. The ratio of occurrence of unrecovered worms in the liver and intestine was 1:14, respectively. Less than one percent of the 91 hamsters perfused were completely free of all schistosomes. This technique recovered 84.2 percent of the 20,914 worms present in these animals. The ratio of occurrence of unrecovered worms in the liver and intestine was 1:10, respectively. About 50 percent of the livers in hamsters were free of schistosomes, whereas the mesenteric veins were rarely free of worms.

DISCUSSION

This equipment afforded a rapid method for recovering large quantities of *S. mansoni* worms from infected rodents. Previously described techniques, in our hands, required a minimum of 15 minutes per animal; using the "Perf-O-Suction" technique only 2 minutes per mouse and 4 minutes per hamster were required. A competent technician was able to sacrifice, harvest, and count the worms collected from a maximum of either 100 mice or 50 hamsters per day. These numbers of perfused animals yielded between 8,000 to 10,000 worms which represented approximately 0.5 g of lyophilized material. The technique was also successfully used to collect schistosomes from rabbits and monkeys. It is recommended that liver and mesentery presses be made periodically on perfused animals to determine the technician's degree of efficiency and his continuing ability to handle the "Perf-O-Suction" technique.

SUMMARY

The basic advantages of the "Perf-O-Suction" technique are:

1. Animals are easily prepared and can be

perfused in groups. Few incisions are required and no ligations are necessary for small animals.

2. A suction apparatus draws the schistosomes into a separatory funnel. Slow, continuous agitation within the funnel prevents congealing of the perfusate and allows the worms to settle to the bottom.

3. An automatic pipetting machine with a foot switch provides pressurized saline for flushing the worms from the liver and mesenteric veins.

4. A skilled technician can perfuse up to 100 mice or 50 hamsters per day, yielding approximately 0.5 gram of lyophilized schistosomes.

REFERENCES

FAUST, E. C. AND MELENEY, H. E. 1924 Studies on schistosomiasis japonicum. Am. J. Hyg. Monog. Series No. 3, pp. 1–339.

PAN, C. AND HUNTER, G. W. III. 1951 A modified perfusion technique for the recovery of schistosomes. J. Lab. Clin. Med. 37: 815–816.

RADKE, M. G., BERRIOS-DURAN, L. A. AND MORAN, K. 1957 A perfusion procedure (Perf-O-Suction) for recovery of schistosome worms. J. Parasit. 43 (supp.): 26–27.

YOLLES, T. K., MOORE, D. V., DE GUISTI, D. L., RIPSOM, C. A. AND MELENEY, H. E. 1947 A technique for the perfusion of laboratory animals for the recovery of schistosomes. J. Parasit. 33: 419–426.

RESEARCH NOTE

EFFECT OF FUMAGILLIN IN MIXED CULTURES CONTAINING *ENTAMOEBA HISTOLYTICA* AND *TRICHOMONAS HOMINIS*.

Many of the media used for the cultivation of *Entamoeba histolytica* will also allow the growth of *Trichomonas hominis*. In Mexico, during the summer of 1960, numerous stool samples from patients with amebiasis were cultured in a medium consisting of a solid phase of egg and blood and a liquid phase of Ringer solution to which rice powder was added immediately prior to inoculation of the media. The media were prepared by blending eggs and whole sheep blood (1 egg to 5 ml of blood), dispensing into test tubes in 5 ml amounts, inspissating in the slanted position for 15 min at 80 C, and then autoclaving the tubes. The solid phase was overlaid with 5 ml of sterile Ringer solution. The tubes were incubated at 37 C overnight to determine sterility of the tubes. Many of the stool samples contained both *E. histolytica* and *T. hominis*, and both grew luxuriously in this medium in the presence of mixed bacterial flora. Attempts to eliminate the unwanted *T. hominis* met with failure. Any treatment which affected the trichomonads also adversely affected the amoebae. Elimination of the amoebae in order to obtain a mono-protozoal culture of *T. hominis* was accomplished by the addition of 10 to 1,000 micrograms per ml of fumagillin to the culture tubes. The amoebae were promptly killed by this highly active amebacidal agent, whereas the trichomonads were apparently unaffected. After several transfers in the presence of fumagillin, amoebae were not observed when the cultures were examined microscopically, whereas the trichomonads flourished. An advantage in using fumagillin is that this agent is not bactericidal; therefore, the bacterial flora of the cultures were not affected, allowing excellent growth of *T. hominis*.

This study was supported in part by a research grant (E-3129) from the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service, to Dr. Francisco Biagi F., Chief, Seccion de Parasitologia, and by a Research Fellowship in Tropical Medicine from Louisiana State University School of Medicine, supported by a training grant (2E-7) from the National Institutes of Health, U. S. Public Health Service, under the direction of Dean William W. Frye.—MITSURU NAKAMURA, *Department of Microbiology, Montana State University, Missoula,* and Seccion de Parasitologia, Unidad de Patologia, Escuela de Medicina, U. N. A. M., Hospital General, Mexico, D. F.

A TWO-COMPONENT APPARATUS FOR CONSTRUCTING COBB METAL SLIDES FOR MICROSCOPE MOUNTS*

PIERRE J. JUTRAS AND A. C. TARJAN
Citrus Experiment Station, University of Florida, Lake Alfred

A necessary adjunct to most microbiological laboratories is an adequate microscopic slide reference collection for use in identification of diverse microorganisms. Standard glass microscopic slides, although adequate for general purposes, present disadvantages among which fragility, weight, and room required for storage are not minor. A very satisfactory substitute for glass slides was proposed by Cobb (1917) for preserved nematode specimens. He illustrated a "special object" slide in which two cover glasses were mounted in a thin metal carrier. Such a slide was quite durable, resisting breakage even when dropped upon the floor. It also enabled the microscopist to examine the mounted specimen from both the top as well as the bottom surfaces of the slide. Then, too, such slides were capable of being stacked for storage directly on one another. Courtney (1936) illustrated two components of an apparatus designed to produce Cobb's "special object" slides from either tin or aluminum. The first component punched a hole through the pre-cut metal blank, while the second component functioned in crimping the sides of the slide.

The unavailability of a dependable source of supply for these slides stimulated development of the slide-making apparatus described in this paper.

The machine is composed of three distinct assemblies (fig. 1 A), from left to right: (a) The aluminum tape dispenser assembly; (b) the punch and die assembly; and (c) the forming tool assembly. The tape dispenser (fig. 1 A, a) holds a standard roll of 1.25 in × .015 in aluminum tape.** This tape is hand-fed into the punch and die assembly (fig. 1 B, a) where the tape is cut into 3-in lengths and a .75-in hole is punched in the center (fig. 1 B, b). There must not be excessive clearance between the punch and the die as this results in the formation of a slight burr at the edges of the hole.

The shearing tool is beveled (fig. 1 C, a) in order to give a gradual sloping cutting action as it decends on the aluminum tape. The pressure required for shearing the aluminum with a beveled tool is approximately one-half the pressure required using a flat tool.

After the first operation is completed, the punched aluminum blank is inserted into the forming tool where two operations occur. First, the upper lever is depressed (fig. 2 A, a). The pressure pad (fig. 2 A, b) introduces the aluminum strip betwen two forming blocks (fig. 2 A, c), and the strip takes the shape illustrated in the foreground (fig. 2 A, d). The upper level is then released and the lower lever is raised (fig. 2 B, a) until the forming blocks meet (fig. 2 B, b). The lower lever is then returned to its original position, completing formation of the slide (fig. 2 B, c).

Microscopic specimens are mounted in preservative between two glass coverslips of No. 0 thickness. The bottom coverslip is 25 mm square, while the top coverslip is circular and 18 mm in diameter. A suitable adhesive material is applied to seal the edges of the smaller coverslip and affix it to the larger coverslip. Once the joined coverslips are placed in the center of the metal slide, rigid vinyl plastic inserts* of .018 in. thickness are introduced from each end of the slide. Such inserts are held sufficiently secure by friction and, if cut to proper dimensions, will position the glass slide firmly in place. The upper white surface of the inserts presents a completely smooth area on which the finest lettering can be inked with facility. Figure 2 C shows the sequence in forming the completed slide. In figure 2 C, c and d note that the crimped sides

Received for publication November 21, 1960.
* Florida Agricultural Experiment Stations Journal Series, No. 1199.

** Roovers-Lotsch Corporation, 3611 Fourteenth Avenue, Brooklyn 18, New York.

* Cut from No. 1937/263 white tree tags, 2 in × 8 in, C. E. Buckley Company, 34 Tremaine Street, Leominster, Massachusetts.

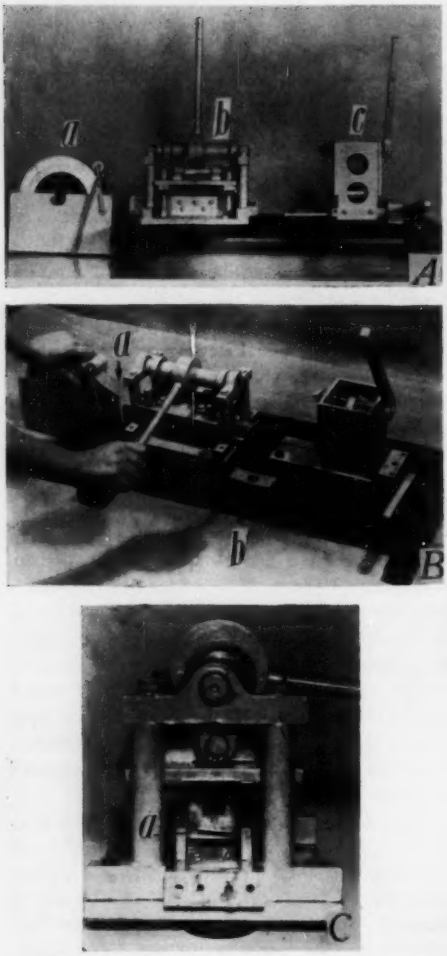


FIGURE 1, A. Apparatus for constructing Cobb metal slides, side view.
a, aluminum tape dispenser; b, punch and die assembly; c, forming tool assembly.
B. Operation of first stage of apparatus.
a, cutting and punching of tape; b, aluminum blank resulting from operation of the first stage.
C. End view of first stage.
a, beveled-edge shearing tool.

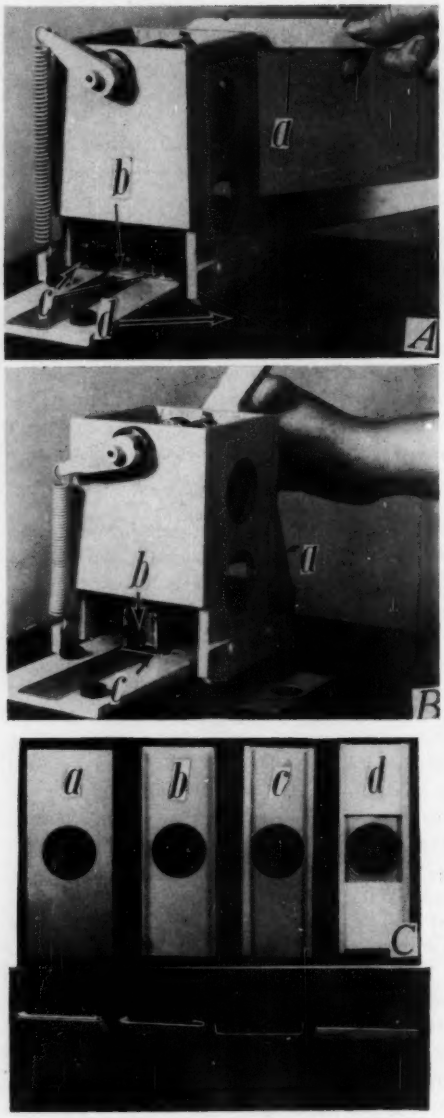


FIGURE 2, A. Second stage of apparatus—operation of pressure pad assembly.
a, pressure pad operation lever; b, pressure pad; c, forming blocks; d, metal slides.
B. Second stage of apparatus—operation of forming block assembly.
a, forming block operation lever; b, forming blocks; c, completed slides.
C. Sequence in constructing slides.
a, punched metal blank resulting from first stage; b, slide with sides bent at 90° angle following pressure pad assembly activation; c, slide with finished crimp following forming tool assembly activation; d, slide equipped with cover glass mount and plastic inserts.

form a more acute angle than in the more conventional metal slide. Not only does this permit proper seating of the vinyl inserts (Fig. 2 C, d) but results in a thinner slide, 100 of which require only 20 cm of storage space.

Plans of this machine will be furnished upon request to the authors.

LITERATURE CITED

COBB, N. A. 1917 Notes on nemas. Intra vitam color reactions in nemas. Contrib. Sci. Nematol. (Cobb) **5**: 120–124.

COURTNEY, W. D. 1936 Metal slide mounts for microscopic objects. Proc. Helm. Soc. Wash. **3**: 72–74.

RESEARCH NOTES

THE DISTRIBUTION OF THE PROGENETIC TREMATODE, *ASYMPHYLODORA AMNICOLAE* STUNKARD, 1959.

Stunkard (1955, Rev. Ibér. Parasit., Tomo Extraordinario, pp. 633–644) described the occurrence of a progenetic trematode, *Asymphylodora*, in the fresh-water snail, *Amnicola limosa*, from ponds near Woods Hole, Massachusetts. Stunkard (1959, Biol. Bull. **117**: 562–581) later described the trematode as a new species, *Asymphylodora amnicolae*, and proved that the entire life cycle can occur in the snail.

During the summer of 1959, a survey was made of the cercariae from aquatic snails of Lake Itasca, Minnesota. Nine of 330 specimens of *Amnicola limosa* were found to harbor sexually mature trematodes, in addition to various species of cercariae. Each of the nine infected snails contained a single unencysted worm. These parasites agree in size and morphology with those described by Stunkard (1959), and are considered to be the same species, *Asymphylodora amnicolae*.

This note represents the second locality record for this species and only the third report of the genus in North America.

This investigation was supported by a National Science Foundation summer fellowship for graduate teaching assistants, 1959.—OMER R. LARSON, *Department of Zoology, University of Minnesota, Minneapolis.*

DEMONSTRATION OF ANTIGEN–ANTIBODY REACTION WITH A MONOBACTERIAL CULTURE OF *ENTAMOEBA HISTOLYTICA* IN AGAR-GEL AND ON CELLULOSE ACETATE MEMBRANE.

A clear-cut demonstration of antigen-antibody reactions with *E. histolytica* as an antigen has been hampered by impure extracts (mixtures of amoebae and biological associates). Further, it has generally been claimed that parasitic amoebae have poor antigenic potency. The present worker has attempted to demonstrate specific antigen-antibody reactions due to amoebae using a monobacterial culture of *E. histolytica*. This brief report describes: (1) A simple and reliable method for the preparation of an antigen extract of *E. histolytica* (*DKB* strain growing with *Clostridium perfringens*); (2) a method for the production of *anti-E. histolytica* serum; (3) a demonstration of antigen-antibody reaction by two independent techniques.

Preparation of antigen: Monobacterial cultures containing *C. perfringens* as an associate were grown in Balamuth's liver-eggyolk infusion medium supplemented with 10 percent beef serum. Amoebae were pooled from cultures (48 hr-old) and then washed six times with 0.9 per-

cent NaCl. Washed amoebae were packed in 0.5 ml of 0.9 percent NaCl and were given 5 minutes oscillating treatment in a Raytheon Magnetostriction Oscillator (50-Watt, 9 K-c Model S-102A).

Preparation of antiserum: 0.5 ml of antigen was mixed with 0.5 ml of Bacto-Adjuvant, Complete (Freund) and in this state the antigen was injected into a rabbit. Two subcutaneous injections were made, 3 weeks apart; approximately 4×10^6 amoebae were used for each injection. Anti-*DKB* serum was obtained 3 weeks after the second injection. (*C. perfringens* accompanying the amoebae did not produce pathology in the rabbit).

Test for antigen-antibody reactions: Two independent techniques were used to test the antigen-antibody reactions: (1) slide-gel diffusion precipitin test as described by Mansi (1958, Nature **181** (4618): 1289–90); and (2) the cellulose acetate paper technique as described by Consden and Kohn (1959, Nature **183** (4674): 1512). Antigen used in the test did not contain any adjuvant. Two controls were used: (1) the pre-immunized serum or the control serum and (2) the antigenic fraction of *C. perfringens*. Antigenic fraction of *C. perfringens* was prepared following the method used in the preparation of antigen of amoebae.

Slide-gel diffusion precipitin test is essentially based on Ouchterlony gel-diffusion technique as described by Oudin (1952, Methods in Medical Research, 5. The Year Book Publishers, Inc., Chicago). Wells of the slides were filled 4 times at intervals of approximately 0.5 to 1 hr. Antigen-antibody reactions in the form of precipitation bands (3 in number) could be seen 4 hours after the last filling of the well. During this whole period the slides were incubated at 30 C in a moist chamber. In cellulose acetate membrane technique 2 microliters of antiserum and 3 microliters of antigen were sufficient to give good results. The cellulose acetate membrane was incubated at 30 C for 20 hrs in a moist chamber and then stained with nigrosin. After the staining the precipitation bands could be observed. This technique gave the same result as was obtained by agar-gel method.

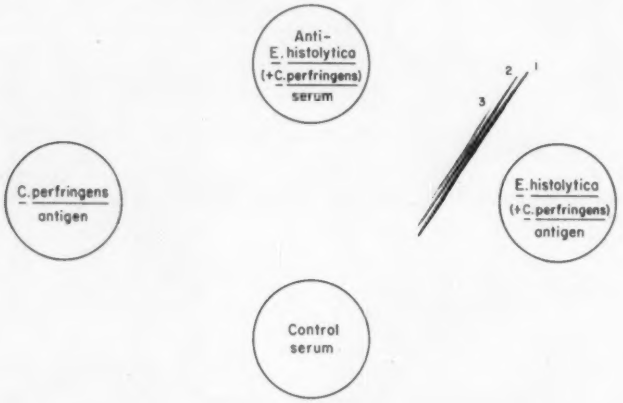


Fig.1

Diagrammatic Representation of Antigen-Antibody Reaction in Agar-Gel

A diagrammatic representation of the result of this experiment is shown (fig. 1). The results indicate that *E. histolytica* has a multiple antigen system. No reaction occurred in two controls, i.e., control serum vs *DKB* or *C. perfringens* antigens and *anti-DKB* serum vs *C. perfringens*.—WASIM A. SIDDIQUI, *Department of Zoology, University of California, Berkeley 4.*

PAPER ELECTROPHORESIS OF SERA FROM MAN AND EXPERIMENTAL ANIMALS INFECTED WITH VARIOUS HELMINTHS*

IRVING G. KAGAN** AND CHAUNCEY G. GOODCHILD***

Parasitologists are finding increasing use for electrophoretic techniques in the analysis of helminth infections (Stauber, 1954). Electrophoretic analysis of serum proteins is also finding increasing application in clinical diagnosis of disease, since pathological and physiological conditions induced by specific diseases alter serum fractions or total amounts of serum proteins in predictable patterns (Wall, 1958). In the following study, the distribution of serum proteins obtained from human beings with echinococcosis, schistosomiasis, and trichinosis was analyzed electrophoretically, as was serum from laboratory animals with experimental schistosomiasis and trichinosis.

MATERIALS AND METHODS

Sixty-six human sera which had been stored frozen were analyzed during the present investigation. These included: 26 sera found serologically positive for trichinosis by the bentonite flocculation test (Norman et al, 1956); 23 sera found serologically positive for echinococcosis with both flocculation and hemagglutination tests (Kagan et al, 1959); 16 sera from patients passing eggs of *Schistosoma mansoni* in their stools; and a Versatol standard (Klein and Weissman, 1958).

Sera from small laboratory animals with schistosomiasis were analyzed. The sera were originally collected for studies of the "CHR" reaction (Kagan and Levine, 1956) and were stored in a frozen state for several years prior to their use. White mice, in two groups of 48 and 26, respectively, were each exposed to 150 to 200 cercariae of *S. mansoni* percutaneously. Two animals were killed approximately every third or fourth day, during an infection period of 97 days, and two, occasionally three, serum samples drawn successively were pooled. Twenty-five young golden hamsters were exposed to 250 to 300 cercariae each and 1 or 2 animals were bled every third day during an infection period of 40 days, and successive serum samples were pooled, as above. Four young guinea pigs were each exposed to 5 to 10×10^3 cercariae. Two animals picked at random were bled from the heart every third or fourth day during an infection period of 87 days, and the two sera pooled. Ten young rats, averaging 107 g in weight, were each exposed to 400 cercariae; groups of four animals were selected at random and bled from the heart every third or fourth day during an infection period of 83 days; two, occasionally three, serum samples were pooled. Three white rabbits, weighing 4 kg each, were exposed to 5,000 (R15), 15,000 (R17), and 80,000 (R20) cercariae of *S. mansoni*, and were bled every third or fourth day from an ear vein for serum; R15 was bled during an infection period of 109 days, R17 during 78 days, and R20 during 89 days.

Three rabbits and 21 rats were infected per os by stomach tube with larvae of *Trichinella spiralis*. Rabbits 29, 30, and 31, weighing approximately 4 kg each, received respectively 12,000, 18,000, and 24,000 larvae. Two of these hosts were bled weekly for serum during infection periods of about 60 days, the third rabbit (R31) died from the infection on the 24th day. Six young rats each received 6,000 *T. spiralis larvae* and were bled during an infection period of 25 days; 2 rats received 2,500 each and were bled during an infection period of 62 days; 3 rats received 1,200 larvae each and were bled during an infection period of 25 days; and, finally, 10 rats received 1,000 larvae each and were bled during an infection period of 41 days. All sera were obtained by intracardial puncture for a serological study (Kagan and Bargai, 1956), and were stored frozen until used in this analysis.

Prior to infection with *S. mansoni* or *T. spiralis*, or within 2 to 7 days following, experimental hosts were each bled to obtain a control serum for that host. These control serums were maintained under the same frozen conditions as were experimental ones.

All sera were analyzed on a Spinco paper electrophoresis apparatus. Ten microliters of serum were applied to paper strips which were then subjected to 2.5 ma of current for 20 hours in an air-conditioned room, at a temperature of approximately 23 C. A Veronal buffer (0.015 M diethyl barbituric acid plus 0.075 M sodium diethyl barbiturate), pH 8.6, ionic strength 0.075, was used. Strips were dried in an oven at 125 C for 30 minutes, fixed in absolute methanol, and stained with 0.1 percent bromphenol blue in absolute methanol (Block, Durrum and Zweig, 1958). Stained strips were rinsed through several changes of 5 percent acetic acid and dried at 125 C.

After intensifying the color with ammonia fumes, strips were placed in a recording densitometer, the Spinco Analytrol, and the pattern of the serum proteins traced on graph paper. The

Received for publication December 15, 1959.

* This investigation was supported (in part) by a research grant (P H S – E 795) from the Institute of Allergy and Infectious Disease, National Institutes of Health, Public Health Service.

** U. S. Department of Health, Education and Welfare, Public Health Service, Communicable Disease Center, Atlanta, Georgia.

*** Department of Biology, Emory University, Atlanta, Georgia.

paper strips were then superimposed on the graphed pattern and the five principal fractions of the serum subdivided by inspection. The percentages of albumin, alpha-1, alpha-2, beta and gamma globulins were calculated by counting the number of saw-teeth on the integrating line of the Analytrol pattern.

All sera from rabbits and rats with experimental trichinosis were analyzed for total protein by the Biuret method (Layne, 1957). In addition, 45 analyses by the Biuret method were made upon serum proteins from human beings: 27 with trichinosis, 5 with echinococcosis, and 13 with schistosomiasis. Biuret determinations for total proteins were also made using the Versatol standard.

EXPERIMENTAL RESULTS

Mean percentage values for serum proteins in the Versatol standard, analyzed by paper electrophoresis, were: albumin, 67.8 ± 1.1; alpha-1 globulin, 3.2 ± .02; alpha-2 globulin, 6.3 ± .03; beta globulin, 9.6 ± .11; and gamma globulin, 13.0 ± .23 (table I). Samples were analyzed on 16 strips, and a very high degree of uniformity was obtained as evidenced by the low S.E. of the mean. The mean A/G ratio for the Versatol standard calculated from the data above is 2.1.

TABLE I. *Electrophoretic analysis of a "Versatol" human standard serum and sera from patients with echinococcosis, trichinosis, and schistosomiasis.*

Serum fractions	Versatol standard	Trichinosis	Echinococcosis	Schistosomiasis
Albumin	67.8 ± 1.1*	64.1 ± 1.5	48.3 ± 3.3	61.3 ± 2.2
Alpha-1 globulin	3.2 ± .02	2.4 ± .15	3.4 ± .54	1.7 ± .17
Alpha-2 globulin	6.3 ± .03	9.9 ± .67	13.6 ± 1.7	6.9 ± .62
Beta globulin	9.6 ± .11	8.6 ± ...8	14.9 ± 1.4	12.8 ± .72
Gamma globulin	13.0 ± .23	15.1 ± 1.3	19.7 ± 1.7	17.4 ± 1.3
No. sera	16**	26	23	16
A/G ratio	2.1	1.8	1.2	1.6

* All figures represent percentages with S.E.M
** Represents one standard examined 16 times.

Percentages of serum proteins in trichinosis, echinococcosis, and schistosomiasis, and a typical pattern for these human diseases are shown in table I and in figure 1, respectively. Individual patterns obtained with trichinosis sera varied slightly, but no major differences in configurations were noted in the samples. Gamma and alpha-2 globulins were higher than in the normal standard; the other fractions were within the range of variation of the standard. An average A/G ratio of 1.8 was obtained. The echinococcosis sera also revealed minor variation within the series. Compared to the standard, albumin was decreased, but alpha-2, beta, and gamma globulins showed large increases. A few individual patterns showed excessive increases in alpha-1 globulin (reaching values as high as 11.5 percent). An average A/G ratio of 1.2 was computed. Patterns with schistosomiasis sera also revealed some deviations from the normal; alpha-1 was decreased, whereas beta and gamma globulins were increased. An A/G ratio of 1.6 was determined.

A composite of the serum protein changes in four experimental hosts with schistosomiasis is illustrated in figure 2. In the mouse, serum albumin decreased from 49 to 32 percent over a period of 90 days; alpha-1 fluctuated between 6 and 23 percent, but tended to show a minor net decrease; alpha-2 remained relatively stable at 20 percent for the first 35 days, beyond which fluctuations between 35 and 11 percent occurred; beta showed a steady increase from 10 to 40 percent; gamma globulin remained relatively stable at 10 percent for the first 50 days, but between 50 and 75 days it increased to 33 percent, after which it decreased. In the hamster, albumin showed a decrease from 57 to 27 percent; alpha-1 fluctuated between 3 and 10 percent; alpha-2 fluctuated around 15 percent for 80 days; beta increased slightly from 10 to 25 percent throughout the 80 days of infection; gamma globulin showed a fluctuating increase from 5 to 20 percent during the first 35 days of infection. In the guinea pig, albumin (45 percent), alpha-1 (10 percent), alpha-2 (23 percent), and beta (12 percent) remained relatively constant during 80 days of infection; however, there was an increase from 10 to 30 percent in the gamma globulin between 32 and 45 days after infection, followed ultimately by a return to 10 perecnt over the next 35 days. In the rat, there was a minor increase in albumin (from 32 to 52 percent) and in alpha-1 (from 5 to 16 percent), and a minor decrease, from 16 to 8 percent, in alpha-2; beta (18 percent) and gamma (10 percent) globulins remained relatively stable during 85 days of infection.

Serum protein patterns for rabbits infected with *S. mansoni* showed some variation during progressive disease. Rabbit 15 revealed sharp increases in alpha-1, from an average of 11 percent

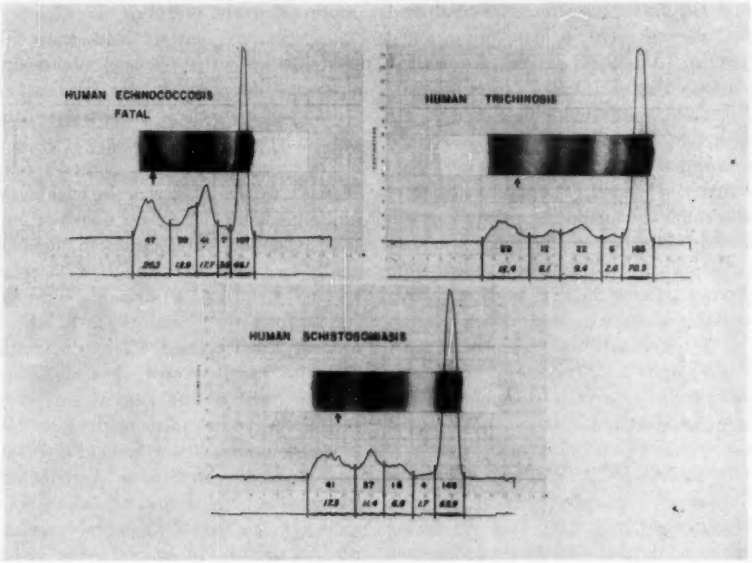


FIGURE 1. Electrophoretic patterns of human sera from three helminth infections. Stained strips superimposed on Analytrol patterns; upper numbers indicate areas, lower numbers percentages.

to approximately 35 percent at 35 and 56 days, respectively, after exposure; albumin fluctuated from an average of 55 percent to lows of 32 percent and highs of 82 percent on about the 35th and 90th days of infection, respectively. The other fractions remained relatively constant. Rabbit 17 revealed a minor increase in beta globulin, from 10 to 21 percent about 50 days after exposure, whereas other globulin fractions remained relative constant; albumin decreased from 72 to 51 per cent about 50 days after exposure. Rabbit 20 revealed a general decrease in albumin, from 75 to 52 percent, during the first 60 days of infection, after which percentage values returned

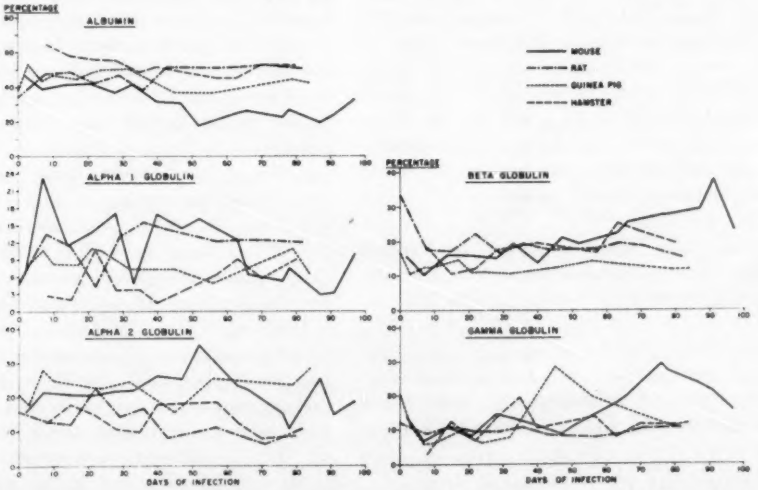


FIGURE 2. Percentages of serum proteins in four experimental hosts infected with *Schistosoma mansoni*.

to normal levels; during the same interval alpha-1 and alpha-2 showed slight increases, from about 5 to 12 percent. Average A/G ratios for these three hosts varied from 1.3 to 2.3.

The data for rats infected with *T. spiralis* are pooled since the number of rats used was small and the duration of infection for individual groups varied considerably. The percentages of individual serum proteins during progressive disease remained fairly constant for over 62 days of infection. Albumin showed a slight increase, from 30 to 40 percent, during the first 15 days, gamma globulin fluctuated slightly, from 15 to 22 percent, 15 to 25 days after infection, but alpha-1 (10 percent), alpha-2 (20 percent), and beta (27 percent) globulins remained at normal levels. Average A/G ratios for all rat hosts varied from 0.4 to 0.7.

The two rabbits (R29, R30) infected with *T. spiralis* showed slight decreases in albumin, from 75 to between 50 and 60 percent, during approximately 60 days of infection, whereas during the same period alpha-1 (5 percent), and alpha-2 (10 percent), and beta (10 percent) globulins remained constant; after 20 days, gamma globulin showed a steady increase from 5 to 25 percent. Rabbit 31, which died on the 24th day, showed greater variation in percent of albumin and globulin fractions. Average A/G ratios for the two rabbits fluctuated between 1.3 and 2.7.

Total serum protein values, determined by the Biuret method, for rabbits and rats infected with *T. spiralis* showed a general rise with progressive disease. Values for R29 varied from 11.5 to 17.4 grams percent, for R30 from 7.3 to 12 grams percent, and for R31 from 6.4 to 8.3 grams percent. Total serum protein values for normal rats varied from 4.6 to 6.4 grams percent. These values increased to 9.2 to 12.8 grams percent after infection. Results are somewhat erratic, but a general tendency toward increased total serum proteins was evident.

Total serum protein values of the Versatol standard showed a mean value of 6.4 grams percent. Analysis of 27 sera from patients with trichinosis gave a mean value of 7.7 (5.9 to 10.1) grams percent of serum proteins; five sera from patients with echinococcosis had a mean value of 7.4 (6.9 to 7.8) grams percent, and 13 sera from patients with schistosomiasis gave a mean value of 8.5 (7.3 to 9.2) grams percent of serum proteins.

DISCUSSION

Successful application of paper electrophoresis as an aid in the diagnosis of helminth disease is still premature, but the data from this limited study indicate that changes observed in serum proteins reflect gross physiological alterations within the host which are correlated to the biology and pathology of the disease.

For each human disease studied a definite trend in patterns was observed. In trichinosis, gamma and alpha-2 globulins were increased, whereas all other fractions were similar to the standard. In echinococcosis, albumin was decreased; but alpha-2, beta, and gamma globulins showed increases from the normal values. In schistosomiasis, alpha-1 was decreased, whereas beta and gamma globulins were elevated. The small size of the samples used in the present studies precludes, at this time, final judgement as to the ultimate diagnostic value of this method of evaluation.

Since uninfected mice showed little or no variation in percentage composition of serum proteins during a period of longer duration than any used in the present study (Evans and Stirewalt, 1957), it was felt that a similar constancy of serum proteins would probably obtain during the relatively brief infections in the animals used in this study. A base line of component proteins in an animal subsequently infected was considered, therefore, as an adequate control for changes induced in that animal during the course of its infection.

In experimental schistosomiasis alteration of protein fractions in the serum of certain host species during progressive disease appeared to be a reflection of the host-parasite relationship. Of five experimental hosts, three (rabbit, guinea pig, and rat) can be termed resistant hosts, whereas two (mouse and hamster) are susceptible hosts. In the two susceptible hosts, beta and gamma globulins both increased steadily during progressive disease, but did not in the three resistant ones. These findings are in general agreement with Evans et al (1955), Evans and Stirewalt (1957), and Sadun and Walton (1958). The time of appearance of increased gamma globulins was directly correlated to the initial appearance of "CHR" antibodies. Antibodies ("CHR") appeared in the mouse approximately 52 days after infection (Kagan, 1955), and in our study gamma globulin showed normal levels until 50 days at which time an increase occurred; in the guinea pig "CHR" antibodies were present only after 34 days of infection, and in our study gamma globulin was first elevated 32 days after infection; in the hamster antibodies appeared 20 days after

infection, and in our study elevation of gamma globulin occurred about 35 days after infection. The rat did not produce "CHR" antibodies, nor was there an increase in gamma globulin.

The serum protein patterns in experimental trichinosis are relatively stable compared to patterns obtained in experimental schistosomiasis. In trichinosis, in the rabbit and rat, albumin, alpha-1, alpha-2, and beta showed little fluctuation, which is a reflection of non-involvement of the liver in this disease. In infections with *T. spiralis* the adults have a transient tissue invasive phase for approximately 2 to 3 weeks, and the larvae are encysted in the muscles relatively quickly after larviposition. In contrast, in schistosomiasis all post-penetrative stages are in intimate contact with the tissues of the host, and there is continuous insult and injury to such vital organs as the intestine and liver. In experimental trichinosis, the total serum protein values increased during infection; but, except for increased gamma globulin, other serum protein fractions remain relatively unaltered.

In hemagglutination and precipitin studies with sera from rabbits infected with *T. spiralis* positive hemagglutination was detected the first week of infection, but precipitin reactions only after 3 weeks (Kagan and Bargai, 1956). These results are of interest because the increase in gamma globulin coincides with the appearance of the positive precipitin test, whereas there are no significant serum changes to correlate with the earlier hemagglutination reaction.

SUMMARY

Sixty-six human sera representing 26 sera found serologically positive for trichinosis, 23 sera positive for echinococcosis, 16 sera positive for schistosomiasis, and a Versatol standard were analyzed by paper electrophoresis. In addition, sera from experimental hosts infected with *Schistosoma mansoni* (mouse, hamster, rat, rabbit, and guinea pig), and with *Trichinella* larvae (rats and rabbits) were similarly analyzed.

Data obtained from this study indicate that for each human disease changes observed in serum proteins reflect physiological alterations within the host conditioned by the biology and pathology of infection. In experimental schistosomiasis with five host species, alteration in serum protein fractions appeared to be correlated with the host-parasite relationships. Susceptible hosts revealed more prompt and more pronounced serum protein changes than did non-susceptible hosts. In experimental trichinosis, increases in gamma globulin and total serum proteins were the only significant changes.

Total serum protein changes were determined by the Biuret method for human trichinosis, echinococcosis, and schistosomiasis.

REFERENCES

BLOCK, R. J., DURRUM, E. L. AND ZWEIG, G. 1958 A Manual of Paper Chromatography and Paper Electrophoresis. Academic Press, New York.

EVANS, A. S., STIREWALT, M. A. AND MACKENZIE, M. 1955 Serologic reactions in *Schistosoma mansoni* infections. II. Cercarial behavior in electrophoretically separated fractions of sera of infected and uninfected mice. Exp. Parasit. **4**: 419–426.

EVANS, A. S. AND STIREWALT, M. A. 1957 Serologic reactions in *Schistosoma mansoni* infections. III. Ionographic fractionation of sera of mice with progressive disease. Exp. Parasit. **6**: 8–17.

KAGAN, I. G. 1955 Studies on the serology of schistosomiasis. I. The in vitro activity of cercariae and miracidia in serum of experimental natural and immunized hosts. Exp. Parasit. **4**: 361–376.

KAGAN, I. G. AND BARGAI, U. 1956 Studies on the serology of trichinosis with hemagglutination, agar diffusion tests and precipitin ring tests. J. Parasit. **42**: 237–245.

KAGAN, I. G. AND LEVINE, D. M. 1956 Studies on the serology of schistosomiasis. II. The in vitro activity of cercariae of *Schistosoma mansoni* in sera of normal and antigen-injected animals. Exp. Parasit. **5**: 48–58.

KAGAN, I. G., ALLAIN, D. AND NORMAN, L. 1959 An evaluation of the hemagglutination and flocculation tests in the diagnosis of echinococcus disease. Am. J. Trop. Med. Hyg. **8**: 51–55.

KLEIN, B. AND WEISSMAN, M. 1958 Study of dialyzed reconstituted dried serum as a chemical chemistry standard. Clin. Chem. **4**: 194–199.

LAYNE, E. 1957 Spectrophotometric and Turbidimetric Methods for Measuring Proteins. *Methods in Enzymology* III *edited by* Colowick, S. P. and N. O. Kaplan. Academic Press, N.Y.

NORMAN, L., DONALDSON, A. W. AND SADUN, E. H. 1956 The flocculation test with a purified antigen in the diagnosis of trichinosis in humans. J. Inf. Dis. **98**: 172–176.

SADUN, E. H. AND WALTON, B. C. 1958 Studies on the host parasite relationships to *Schistosoma japonicum*. II. Quantitative changes in the concentration of serum proteins in humans and rabbits. Am. J. Trop. Med. Hyg. **7**: 500–504.

STAUBER, L. A. 1954 Application of electrophoretic techniques in the field of parasitic diseases. Exp. Parasit. **3**: 544–568.

WALL, R. L. 1958. The use of serum protein electrophoresis in clinical medicine. A. M. A. Arch. Int. Med. **102**: 618–658.

STUDIES ON THE HELMINTHS OF NUTRIA, *MYOCASTOR COYPUS* (MOLINA), IN LOUISIANA WITH CHECK-LIST OF OTHER WORM PARASITES FROM THIS HOST

BERT B. BABERO AND J. WARREN LEE
Department of Biology, Southern University and A. and M. College, Baton Rouge, Louisiana

Nutria are considered to be of potential economic importance. Although these animals are primarily indigenous to South America (principally Argentina, Brazil, Paraguay, and Uruguay), they have been introduced into parts of Europe and the United States. Adams (1956) lists 18 states in which nutria are known to be feral. In Louisiana, nutria were first introduced about 1937 by E. A. McIlhenny, having been imported from Argentina to Avery Island off the south coast of Louisiana (Ensminger, 1955). Today, it is estimated that there are over 1 million of these animals inhabiting Louisiana marshes. The importance of nutria to agriculture and conservation also is discussed by Ensminger. Except for the limited report by Schacher (1955) and a few miscellaneous references, including those by Hall (1916, 1919), little information is available concerning the endoparasites of this host in North America. Most of the knowledge on the helminths of nutria, especially those organisms which may be capable of decimating their population, has resulted from investigations by Europeans and South Americans. This paper presents some ecological data on the occurrence and prevalence of endoparasites in Louisiana nutria and a check-list of the helminths previously reported from this host.

MATERIALS AND METHODS

The 56 nutria upon which this study is based were collected by trappers from fresh-water marshes of two general localities, Ramah and Melville, Louisiana, from October 1959 to January 1960. Although the majority of the animals were adults, some were judged to be immature (age, 3 months or less) and subadults (age, 3 to 5 months). Most carcasses were stored in a freezer prior to necropsy. About one-fourth of them were examined soon after they were obtained. All helminths were fixed in A.F.A. (Alcohol-formalin-acetic acid) solution. Temporary mounts of nematodes were studied after being cleared in glycerin or lactophenol. Trematodes and cestodes were stained in Semichon's acid carmine or aqueous alum cochineal and mounted for later morphological study.

Received for publication June 15, 1960.

RESULTS AND DISCUSSION

In the present investigation of nutria, a total of 11 helminth species, including as many genera, were recovered. The parasites obtained comprise five species and five genera of Trematoda, one species of Cestoda, one of Acanthocephala, and four species and four genera of Nematoda. Of the animals infected, 3 harbored one helminth species; 27, two species; 11, three species; 8, four species; and 6 were negative. A brief discussion of each parasite follows:

TREMATODA

Heterobilharzia *sp.*
(Figures 1–2)

Fragmentary parts and entire specimens of males and females of this genus were collected from the intestinal contents of three nutria. The flukes were not recognized as schistosomes at the time of necropsy and examinations of blood vessels for additional specimens were not made. The worms apparently entered the intestinal material upon the mechanical rupture of the mesenteric or portal blood vessels. Three entire females, two entire males, and three fragmentary male specimens, with about one-fourth of the anterior end missing, were collected. Microscopical examinations of the trematodes were made before and after permanently mounting. The routine trematode-technique employed in preparation for mounting was unsatisfactory for the schistosome material, since it caused slight shrinkage of the worms and partial collapse of most eggs within the uterus.

A search of parasitological literature revealed that *Heterobilharzia americana* Price, 1912, is the only previously described species of the genus. Since the original description and redescription of the species by Price (1929, 1943)

from *Lynx rufus floridanus* and *Procyon lotor*, only one additional occurrence record has appeared in literature, the report by Miller and Harkema (1960) based upon material collected from *Lynx rufus* in North Carolina. Schistosomes of the genus *Heterobilharzia* from nutria show close resemblance to the type species. However, the number of testes in *H. americana* ranges from 60 to 84, whereas in the nutria worms the range is 35 to 45. The size of testes in the writers' material also is considerably larger than those of the type species. In addition to the features discussed, the nutria schistosomes have a smaller egg-size and spines or tubercles were not seen. Spines are present on *H. americana*. It was pointed out by Dr. McIntosh, Beltsvile Parasitological Laboratory,

TABLE I. *Check-list of major references on helminths of nutria*, Myocastor coypus, *1916–1959**

Parasite	Reported by	Year
TREMATODA		
Chiostichorchis mypopotami	Artigas and Pacheco	1933
"Darmtrematode"	Freund	1930
Dicrocoelium lanceatum	Sprehn	1932
Fasciola gigantica	Akhumian	1956
F. hepatica	Hülphers	1933
	Lorber	1932
	Wolffhügel	1916, 1919
	Lahille	1918
	Schönenberger	1933
****Hippocrepis fuelleborni*	Henry	1931
	Travassos and Vogelsang	1930
"Leberegel"	Gmelner	1931
Notocotylus sp.	Sprehn	1932
Paramphistomum sp.	Gebauer	1932
Stichorchis waltheri	Sprehn	1932
	Grieder	1937
**Schistosoma mansoni* (expt'l.)	Schacher	1955
CESTODA		
****Andrya* (S. lato) sp.	Spasskii	1951
Anoplocephala sp.	Claussen	1933
"Cestode" sp.	Heidegger	1931
Coenurus serialis	Henry	1931
Cysticercus taeniaeformis	Hohner	1954
**Cysticercus* sp.	Schacher	1955
C. talpae	Hohner	1954
"Darmcestode"	Freund	1930
Echinococcus	Sprehn	1930
E. granulosus	Coutelen, Callot and Desportes	1939
E. polymorphus	Henry	1931
E. uniloculoris	Wolffhügel	1951
Hymenolepididae	Akhumian	1956
Hymenolepis octocoronata	Schönenberger	1933
	Gmelner	1931
	Hülphers	1933
	Heidegger	1931
	Meggitt	1924
	Henry	1931
Multiceps sp.	Meggitt	1924
M. clavifer	Pachenstecher	1877
	Railliet and Mouguet	1919
	****Shul'ts	1931
M. serialis	Meggitt	1924
	Hall	1919
	Clapham	1942
Polycephalus serialis	Sprehn	1932
Rodentolepis avetjanae	Akhumian	1956
Schizotaenia sp.	Sprehn	1932
Taenia echinococcus	Wolffhügel	1920
NEMATODA		
Bohmiella perichitinea	Gebauer	1932
Capillaria hepatica	Vogelsang and Espin	1949
Dipetalonema sp.	Gmelner	1931
D. travassosi	Artigas and Pacheco	1933
Filaria kitti	Grieder	1937, 1938
Heligmosomoides polygyrus	Lorber	1932
	Gebauer	1932
	Heidegger	1931
	Schmid	1933
Heligmosomum sprehni	Enigk	1933
H. alpha	Henry	1931
Kittia myocastoris	Sedlmeier	1931
Longistriatia maldonadoi	Artigas and Pacheco	1933
***L. nutria*	Travassos	1937
*****L. myopotami*	Petrov and Sadykhov	1959
Oxyuris hamata	Hall	1916
Strongyloides	Claussen	1933
	Hulphers	1933
	Reinisch	1940
	Gebauer	1932
	Lorber	1932
	Schmid	1933
S. chapini	Sandground	1925
	Enigk	1933
Strongiloides (sic) *myopotami*	Pacheco and Artigas	1932
Strongyloides myopotami	Zajicek	1955
S. papillosus	Sprehn	1932
*****S. ratti*	Erhardova	1958
***Toxocara canis* (expt'l.)	Schacher	1955
Subulura linstowi	Sprehn	1932
"Trichina"	Rubli	1936
Trichocephalus sp.	Claussen	1933
Trichuris myocastoris	Enigk	1933
	Akhumian	1956
	Lent and Teixeira de Freitas	1936
	Zajicek	1955
	Schulz and Landa	1934
	Swierstra, Jansen and Broek	1959
Trichostrongylus sp.	Schönenberger	1933
	Claussen	1933
*****T. colubriformis*	Erhardova	1958
T. retortaeformis	Enigk	1933
"Trichuren"	Gebauer	1932
*****Trichuris opaca*	Henry	1931
Trichuris sp.	Gmelner	1931
	Lorber	1932
	Schmid	1933
	Freund	1930
	Hülphers	1961
	Sprehn	1933
	Schönenberger	1932
	Adams	1933
***Viannaia* sp.	Gebauer	1932
	Schacher	1955

* This list to the writers' knowledge is complete, most records having been taken from the host section of the Index-Catalogue of Medical and Veterinary Zoology, which is maintained in the Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, U.S.D.A., Beltsville, Md.

** Record taken from the report by Schacher.

*** Record taken from Travassos (1937).

**** Record supplied by Dr. A. McIntosh, Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, Beltsville, Md.

U.S.D.A., Beltsville, Maryland (personal communication), that perhaps most of the spines on the writers' material were lost in handling and that a few typical spines, not observed by the writers, could still be seen. Also, according to McIntosh, immature specimens could have fewer and more variable-sized testes than mature worms. He, therefore, suggested that the nutria *Heterobilharzia* belongs to the species *americana*. While agreeing with McIntosh in his morphologic viewpoints, since doubt exists on specific identification, the writers feel that, rather than assigning the nutria worms to a species and having them overlooked in parasitological nomenclature and systematics, it would be preferable to designate them tentatively as *Heterobilharzia sp.* until additional specimens are collected and studied.

Measurements given throughout this paper are in millimeters unless otherwise stated.

Specific diagnosis. Heterobilharzia: (Male) Body 10.5 long. Cuticular spines or tubercles (?) absent i.e., not observed by the writers. Oral sucker 0.31 in diameter. Acetabulum pedunculated, 0.41 wide, situated 0.56 from oral sucker. Esophagus 0.52 long bifurcating at anterior rim of acetabulum to form intestinal ceca which unite at posterior end of body to form common cecum. Genital pore in body-midline, 0.41 from posterior rim of acetabulum. Cirrus pouch elongated containing distinct seminal vesicle, about 0.13 long by 0.08 wide, situated 0.34 from acetabulum. Ovoidal testes in posterior fourth of body, 35 to 45 in number; average size, 0.03 to 0.04. Excretory bladder short. Excretory pore at tip of body. (Female) Body about 16.5 long; maximum width of preovarial portion 0.10, of ovarial portion 0.41 of postovarial portion 0.82. Oral sucker 0.06 in diameter; acetabulum about 0.17 wide, 0.29 from anterior end of body. Esophagus 0.25 long, in part surrounded by unicellular glands, bifurcating near anterior rim of acetabulum. Intestinal ceca in postovarial part of body with diverticula, posterior union not clearly discernible. Genital aperture immediately posterior to acetabulum. Uterus long and slightly tortuous, extending from ootype to genital aperture. Mehlis' gland present. Seminal receptacle bi-lobed, 0.12 long by 0.07 wide. Laurer's canal present. Ovary pre-equatorial, looped. Vitellaria extensive, dispersed anteriorly from seminal receptacle to near end of body. Vitelline reserve postovarial, pronounced with distinct long duct extending cephalad to ootype. Eggs thin-shelled, subspherical to oval; averaging 0.48 in length and 0.36 in width.

Host: Myocastor coypus bonariensis (E Geoffroy-Saint-Hilaire).

Location: Mesenteric or portal vein.

Locality: Ramah, Louisiana.

Specimens: U.S.N.M. Helm. Coll. Nos. 39420 and 39264.

Unidentified Schistosome
(Figure 3)

A single specimen of a schistosome was collected from the intestinal contents of a nutria from Melville, Louisiana. The worm apparently came from one of the ruptured intestinal blood vessels. Employing the keys to the Schistosomatinae Stiles and Hassall, 1898, as presented by Yamaguti (1958) and Price (1929), the writers were not able to find a satisfactory genus for this parasite. In testis number, the specimen obtained from nutria shows some affinity to *Bivitellobilharzia* Vogel and Minning, 1940. This genus, however, is characterized in having the testes disposed in a different manner from the nutria worm. In disposition of testes, the genera *Microbilharzia* Price, 1929, and *Austrobilharzia* Johnston, 1917, show some resemblance to the unknown schistosome, but the testis number does not agree. The genus *Ornithobilharzia* Odhner, 1912, seems a likely category for the inclusion of the Louisiana schistosome, but the disposition of the testes and the tendency of the intestinal ceca to anastomose are charateristics that do not fit the trematode from nutria. Therefore, the parasite is designated an unknown schistosome, pending the collection of additional material. However, a brief description of the worm is included.

Diagnosis: Schistosomatinae: (Male) Body slender, tapering gradually from mid-body region to posterior end, about 6.3 long with a maximum width of 0.66. Cuticle without spines or tubercles. Gynaecophoric canal beginning 0.21 from posterior rim of acetabulum and extending to posterior end of body. Oral sucker 0.29 in diameter. Acetabulum about 0.34 wide. Genital pore apparently near right postero-lateral margin of acetabulum. Cirrus sac either absent or not well developed. Seminal vesicle distinct, ovoidal, near posterior of acetabulum. Esophagus long, posterior half surrounded by unicellular glands, bifurcating just at anterior level of acetabulum. Ceca extending caudally to posterior end of body where they seem to unite forming a common cecum. Testes extra-cecal, appear to be arranged in 2 lateral rows; approximately 24 testes discernible in 1 row.

Host: *Myocastor coypus bonariensis.*

A slide (No. 39421) containing a whole mount of this trematode has been deposited in the Helminthological Collection of the United States National Museum.

Schacher (see table I) pointed out that nutria could be experimentally infected with *Schistosoma mansoni* Sambon, 1907; however,

schistosomes were not recovered from nutria during his helminthological survey because he wrote: "No evidence has been found in this study to support any fear that *Schistosoma mansoni*, an extremely pathogenic parasite of man, could become endemic within the nutria population of this state. This parasite is extremely fastidious in its requirements for propagation, and until the presence of good snail intermediate hosts can be proven to exist side-by-side with a fair concentration of nutria, the question is mainly of academic interest." The findings in the present study reveal that Louisiana nutria do harbor schistosomes and that suitable snail intermediate hosts must be present.

Echinostoma revolutum *Fröhlich, 1802*
(Figures 4–5)

Nine nutria, or 6.2 percent, were infected with this species. The range of infection was 1 to 92 echinostomes per host. The parasites recovered from most infected animals were immature. Three nutria, however, harbored mature worms with lengths ranging from 9 to 16. Nutria apparently represent a new host record for *E. revolutum* (table I).

Limited damage has been attributed to *E. revolutum*. It is known that this parasite may be attached by its circlet of spines (fig. 5) to the intestinal mucosa, producing local irritation. It is highly probable that in heavy infections, such as were encountered in three instances in this study, serious pathological lesions could eventually result.

Cercaria of *E. revolutum* may encyst in the same snail. It is conceivable that nutria may become infected by the ingestion of these intermediate hosts. Snails of the genus *Heliosoma* were occasionally observed in the digestive tract of nutria.

Psilostomum *sp.*
(Figure 6)

Nine nutria (16.4 percent) from Ramah, Louisiana, were observed to harbor metacercaria in their stomachs and small intestines. The range of infection was 3 to over 500 cysts per host. The larval flukes were in a slightly greenish transparent encasement. Although most larvae did not appear to be fully developed, they were fed to two pups, four white laboratory rats, and one rabbit. Adult flukes were not recovered from these hosts upon later examination.

Because of the incomplete development of the trematodes, specific identification of them could not be definitely ascertained. The worms undoubtedly belong to the subfamily Psilostominae Lühe, 1909, and, following Yamaguti's key to genera of this group, they are tentatively assigned to the genus *Psilostomum* Looss, 1899, although they also show some resemblance to *Pseudopsilostoma* Yamaguti, 1958. It is interesting to note that the senior investigator recently has recovered psilostomes from skunks on numerous occasions. A description of the nutria worms is included.

Diagnosis: Psilostomatinae: Total length of body 3.00; maximum width, 0.74. Oral sucker slightly larger than acetabulum, 0.25 by 0.28. Prepharynx and esophagus apparently absent. Pharynx strongly developed, 0.08 by 0.10. Acetabulum spherical, in anterior half of body immediately posterior to fork of intestinal ceca, 0.25. Ovary horizontally elongated, 0.06 by 0.11. Testes tandem; anterior testis 0.27 by 0.34, posterior testis immediately behind anterior one, 0.28 by 0.29 and 0.32 from end of hind body. Uterus intercecal in field between anterior testis and acetabulum. Genital pore just anterior to acetabulum. Cirrus sac extending from genital pore to a level near middle of acetabulum. Seminal receptacle may be present. Vitellaria mostly extracecal but confluent medially in posterior part of body, reaching from level near middle of acetabulum to posterior end of body.

Host: *Myocastor coypus bonariensis*

Metacercariae of this fluke have been deposited in the Helminthological Collection of the United States National Museum (Nos. 39266 and 39271).

Unidentified Aspidogastrid
(Figure 7)

A single aspidogastrid trematode was taken from the small intestine of a nutria. Internal morphology of the fluke was not studied; however, externally the worm resembled certain members of the genus *Aspidogaster* v. Baer, 1827, as figured by Eckmann (1932). Comparative descriptions of species of this genus, as well as of *Lophotaspis* Looss, 1902, are given by Ward and Hopkins (1931), who considered four (possibly five) species as comprising the genus *Aspidogaster*. Dawes (1946) states that only three species of this genus occur in North American hosts (lamellibranch molluscs and fishes); viz, *A. conchiocola* v. Baer, 1827, *A. limacoides* Diesing, 1835, and *A. decatis* Eckmann, 1932. Superficially and in size the para-

site obtained from nutria appears to resemble the latter species. The aspidogastrid collected from this host probably was an accidental infection, since such animals occassionally feed on fish, as indicated by etenoid scales found in the gastrointestinal tract. This indication of feeding habit is in contrast to the observations of Atwood (1950), who did not find any evidence of animal food in the stomach and scat contents of nutria.

CESTODA

Anoplocephala *sp.*

Although about seven species of tapeworms have been previously reported from nutria, in the present study cestodes were not prevalent in this host. An incomplete strobila and the scolex and neck region of an anoplocephalan parasite, respectively, were recovered from 2 nutria of the Ramah area. These fragmentary worms tentatively are identified as *Anoplocephala* sp. Claussen (1933) also reported *Anoplocephala* Blanchard, 1848, from nutria.

ACANTHOCEPHALA

Neoechinorhynchus *sp.*
(Figures 8–9)

Three juvenile specimens of this genus were recovered from the small intestine of two nutria. Since the worms were sexually immature, details of the reproductive tract could not be determined for specific identification. There appears to be no previous record of the occurrence of an acanthocephalan parasite in nutria. Worms of the genus *Neoechinorhynchus* (Van Cleave, 1939) primarily are found as adults in fishes and turtles. The occurrence of this parasite in nutria is believed to be an accidental infection.

NEMATODA

Trichostrongylus sigmodontis *Baylis, 1945*
(Figures 13–16)

Four to 12 specimens of *Trichostrongylus* Looss, 1905, per host were collected from the small intestine of 6 nutria. From this host were previously reported *Trichostrongylus* sp. by Schönenberger (1933) and Claussen (1933) and *T. retortaeformis* (Zeder, 1800). The Louisiana trichostrongyles do not fit the description of *T. retortaeformis*. They do, however, show close similarity to *T. sigmodontis* originally described by Baylis (1945) from the cotton rat (*Sigmodon hispidus*), and *T. ransomi*, described by Dikmans (1937) from a rabbit (probably *Sylvilagus floridanus alacer*). In the latter parasite species, the spicules are shorter, equal, and possess three projections. Also the dorsal ray, after bifurcating, apparently is not bi-digitate. In both *T. sigmodontis* and the nutria trichostrongyles the spicules are unequal and contain 4 to 5 barbs, and the dorsal ray is bi-digitate. Because of the tight inwardly rolled nature of the bursa and the apparently few specimens of both sexes of *T. sigmodontis* available to Dikmans, the pattern of the rays was not discussed. The females of the species were young adults with few fully formed eggs. The writers collected mature worms of both sexes. Several males were sacrificed in order to determine the bursal pattern. A redescription of the species based upon the nutria material is given.

Specific diagnosis. Trichostrongylus: (Female) Body attenuated anteriorly, 3.95 to 4.86 long; width at anterior end about 0.01, at valvular region, 0.07 to 0.08. Esophagus long and slender, 0.68 to 0.70 in length. Lips not well defined. Nerve ring and excretory pore in region 0.09 to 0.10 from anterior end. Vulva in last one-fourth of body 0.68 to 0.85 from tip to tail. Lips of vulva slightly salient. Combined length of ovejectors 0.35 to 0.44. Uteri divergent, posterior extension about 0.13 to 0.15 from posterior end; anterior extension about 1.61 from cephalic end. Eggs in uteri embryonated, averaging 0.07 long by 0.03 wide. Anus 0.07 to 0.09 from tip of tapered tail. (Male) Total length of body 3.20 to 3.29; width at bursa, 0.08 to 0.11, Lateral lobes of bursa well developed, tightly rolled inward. Dorsal lobe not well developed. Medio-, externo-, and latero-ventral rays close, thick, and slightly divergent at tips. Postero-lateral and ventro-ventral rays slender, each arising separately, the former being slightly shorter than latero-ventral ray curving somewhat toward body. Externo-dorsals long and slender, arising from dorsal and extending to base of bursa. Dorsal ray originating slightly asymmetrically from base of externo-dorsal ray, bifurcates giving rise to two slender branches each longer than stem and bi-digitating at tip. Spicules brownish-yellow, twisted, and slightly unequal in length. Right spicule 0.14 to 0.15 long; left spicule about 0.16 long. Approximately 4 to 5 ventral barbs present at posterior end of each spicule. Gubernaculum boat-shaped in profile, posterior end coming to sharp point, averaging 0.08 in length.

Host: Myocastor coypus; Sigmodon hispidus

Unmounted male and female specimens of this species have been deposited in the Helminthological Collection of the United States National Museum (Nos. 56656 and 39272).

Schacher in his report suggested that the specimens of *Trichostrongylus* sp. collected in his study may be *T. calcaratus*. It is probable that his specimens are cospecific with *T. sigmodontis*.

Longistriata maldonadoi *Artigas and Pacheco, 1933*

(Figures 10–12)

Longistriata maldonadoi was the most prevalent and abundant parasite encountered in this study, being recovered from 41 or 73.2 percent of the animals necropsied. The range of infection by this nematode was 4 to 1500 per host. In situ, the parasite is coiled in a loose spiral and may appear red due to the presence of hemoglobin. The red color, however, disappears upon fixation. Microscopically, the worm appears irridescent due to the refraction of light by the closely-set transverse striations. *Longistriata maldonadoi* lives primarily in the small intestine, but occasionally it is found in the large intestine.

Three species of *Longistriata* Schulz, 1926, have been reported previously from nutria; viz, *L. nutria* Gilbert, 1931, *L. myopotami* Petrov and Sadykhov, 1959, and *L. maldonadoi*. The Louisiana specimens fit the description of the latter species as described by Artigas and Pacheco (1933). Travassos (1937) considered *Heligmosomum polygyrus* Heidegger, 1931, and *H. sprehni* Enigk, 1933 (table I) to be synonyms of *L. maldonadoi*. M. B. Chitwood, Animal Parasite Laboratory, U.S.D.A., Beltsville, Maryland (personal communication) stated: "Even the Skrjabin volumes consider *L. nutria* a synonym." Therefore, *L. maldonadoi* and *L. myopotami* appear to be the valid species of the genus previously reported from nutria. The writers have not had the opportunity of seeing the original description and figures of the latter species.

Trichostrongylus sp. figured and described by Claussen (1933) rightfully belongs to the genus *Longistriata*. From the general description of *Viannaia* sp. and the prevalence and abundance cited for the parasite, it is highly probable that Schacher misidentified his specimens, which also actually belong in this genus.

Members of *Longistriata* are common parasites of rodents. Hugghins (1951) stated that all members of the subfamily Heligmosominae Travassos, 1941, probably have a "typical hookworm life cycle," and probably enter the host by penetrating the skin. The fossorial tendencies of nutria and their occasional use of ground burrows and tunnels within levees, dikes, and ditchbanks enhance the chance of their acquiring massive infections with *L. maldonadoi*.

Strongyloides myopotami *Artigas and Pacheco, 1932*

(Figures 17–19)

This minute, white thread-like nematode was the second most prevalent parasite recovered from nutria, having been found in 35 or 62.5 percent of the animals examined. The range of infection was 2 to 42 worms per host. Since the females agree with the description of *S. myopotami* as given by Artigas and Pacheco (1932) they are tentatively assigned to this species. Male specimens were not found, despite our special efforts to look for them. It may be conjectured that the absence of males of *S. myopotami* was due to the selected mode of development by the parasite, since the life cycles of the strongyloidids are unique.

Hall (1916) points out that *S. papillosus* (Wedl, 1856) has 3 possible alternatives: (1) The parasitic female may eventually produce infective filariform larvae to cause autoinfection; (2) after passing from the body of the host, the hatched rhabditiform larvae transform into infective filariform larvae, thus omitting the free-adult stage; (3) the parthenogenetic parasitic female produces within the body of the host rhabditiform larvae which pass from the body with the feces and later give rise to mature free-living male and female worms which, after mating, produce rhabditiform larvae that later transform into filariform larvae and in the body of the host develop into parthenogentic females. Baylis (1936), after discussing certain theories concerning factors which may influence a given mode of development of *Strongyloides stercoralis* (Bavay, 1876), concludes that the controlling factors are still somewhat obscure and imperfectly understood. In the present strongyloidid infections in nutria, it is possible that the latter mode of development was selected, since only female worms were recovered.

In addition to the report by Artigas and Pacheco, members of the genus *Strongyloides* Grassi, 1879, have been reported from nutria

on several occasions; *Strongyloides chapini* by Enigk (1933), *S. papillosus* by Sprehn (1932), *S. myopotami* by Zajiček (1955) and Schacher (1955), and *Strongyloides* sp. by several investigators (table I). It is possible that only one member of the genus occurs in nutria.

Artigas and Pacheco stated that *"Strongiloides myopotami"* is believed to be of pathological significance. However, in the present investigation no evidence of pathology was observed that could directly be attributed to this parasite. Enigk (1950, cited by Schacher) observed that prenatal infection of litters of nutria could occur if the female parent was parasitized 3 weeks prior to parturition. The significance of this observation is that heavy infection of young nutria could seriously retard the growth and development of these animals and may possibly prove fatal.

Trichuris myocastoris *Enigk, 1933*

Trichuris myocastoris was the third most common parasite found in this study. This whipworm was collected from the cecum of 28 or 50 percent of the animals examined. The range of infection was 1 to 74 worms per host. Although some variation was observed in the spicular sheath, the morphology of the Louisiana whipworm closely agrees with the redescription of the species as given by Lent and Freitas (1936). The spicular sheath was observed to be either campanulate, cylindrical, or both (figs. 20–23). Furthermore, the degree of extension of the sheath showed much variation.

There has been much controversy concerning the nomenclature of the whipworm occurring in nutria. The parasite has been reported under numerous names (table I). Lent and Freitas considered *Trichocephalus myocastoris* Heidegger, 1931, *Trichuris* sp. Sprehn, 1932, *Trichuris myocastoris* Enigk, 1933, and *Trichocephalus nutria* Schulz and Petrow *in* Schulz & Landa, 1934, to be synonyms of *T. nutria*. However, these investigators were somewhat confused as to whom authority for the species was to be ascribed. Schulz and Landa in 1934 presented a "redescription" of the parasite and credited the species to Schulz and Petrow, 1935. Thus, Lent and Freitas wrote, "Não é possivel, entretanta, em 1934 referir uma publicacão de 1935. Os auctores russos teem muito o habito de fazerem citacões deste type; acreditamos tratar-se de indicações de trabalhos em preparo ou já em manuscripto ou impressão, o que não podemos levar em consideracõa". In order to clarify the authorities for the species, Lent and Freitas wrote: *"Trichuris nutria* (Schulz & Petrow *in* Schulz and Landa, 1934)." The present writers have searched in vain for the indicated paper by Schulz and Petrow, 1935. We feel that no such paper has been published. Our search shows that *Trichuris myocastoris* Enigk, 1933, has priority and is the valid name for the species.

Although no pathological conditions associated with infections were observed in this study, whipworms are considered to be pathogenic to their host. Concerning *T. suis* (Schrank, 1788), Spindler (1942) stated that massive infections noticeaby retard growth and lower the vitality of young swine and may eventually cause death. Belding (1936) stated that *T. trichiura* (Linn., 1771) may embed its anterior end into the intestinal mucosa and liquefy the neighboring cells and, occasionally, may penetrate the submucosa and muscularis and perforate the peritoneum producing a serious inflammatory condition. It is highly possible that massive infections with *T. myocastoris* do considerable damage to young nutria.

SUMMARY

Fifty-six nutria from 2 areas within the state of Louisiana were examined for their helminth parasites. From these hosts the following worms were collected: TREMATODA—*Heterobilharzia* sp., unidentified schistosome, unidentified aspidogastrid, *Psilostomum* sp., and *Echinostoma revolutum;* CESTODA—*Anoplocephala* sp.; ACANTHOCEPHALA—*Neoechinorhynchus* sp.; NEMATODA — *Trichostrongylus sigmodontis, Longistriata maldonadoi, Strongyloides myopotami,* and *Trichuris myocastoris.*

Information has been presented relative to the occurrence, prevalence, and, in several instances, the possible mode of transmission of the parasites. *Heterobilharzia* sp., an unidentified schistosome and aspidogastrid, *Psilostomum* sp., *E. revolutum, T. sigmodontis,* and *Neoechinorhynchus* sp. comprise new parasite records for nutria. A redescription and some descriptions of parasites were given.

Several parasite-species recovered from nutria are known to be pathogenic to their host; however, pathology that could be attributed to helminth parasitism was not observed in the

animals examined.

ACKNOWLEDGMENTS

The writers are indebted to all persons who assisted in this study. Special acknowledgments are accorded to Dr. Donald K. McLoughlin, Mrs. Maybelle Chitwood, and Dr. Allen McIntosh, Beltsville Parasitological Laboratory, A.R.S., U.S.D.A., Beltsville, Md., for their valuable comments during this study, compiling, and supplying the writers with helminth records from nutria and reviewing the manuscript. Specimens of *Heterobilharzia* also were graciously loaned by Dr. McIntosh, and Mrs. Chitwood kindly made or confirmed the identification of nematodes. The writers also thank Mr. William C. McCall, Department of Biology, Southern University, for assistance in necropsies, and Mr. Charles R. Shaw, Louisiana Wildlife and Fisheries Commission, for supplying study reports on Louisiana nutria.

LITERATURE CITED

ADAMS, A. R. D. 1933 Report on a collection of nematodes from the Federated Malay State. Ann. Trop. Med. Parasit. **27**: 1–13.

ADAMS, W. H. 1956 The nutria in Coastal Louisiana. Proc. La. Acad. Sc. **20**: 28–41.

AKHUMIAN, K. S. 1956 A new cestode—*Rodentolepis avetjanae* nov. sp. from *Myocastor coypus*. Dokl. Akad. Nauk. Armiansk. SSR **22**: 181–191.

ARTIGAS, P. T. AND PACHECO, G. 1932b *Strongyloides myopotami* n.sp. (Nematoda). Compt. Rend. Soc. Biol. **112**: 406–407.

———. 1933a *Chiostichorchis myopotami* (Artigas e Pacheco, 1932), parasito do *Myocastor coipus*. Chiostichorchis n. gn. (Trematoda: Paramphistomidae) [English summary] Rev Biol e Hyg. S. Paulo **3**: 103–111.

———. 1933b *Longistriata maldonadoi* n. sp. (Nematoda), Trichostrongylide parasite du *Myocastor coipus*. Compt. Rend. Soc. Biol. **112**: 1004–1006.

———. 1933d A new species of filaria from *Myocastor coipus*, *Dipetalonema travassosi* n. sp. (Nematoda: Filarioidea). Rev. Biol. e Hyg., S. Paulo **4**: 23–27.

ATWOOD, E. L. 1950 Life history studies of nutria, or coypu in Coastal Louisiana, J. Wildl. Mgt. **14**: 245–249.

BAYLIS, H. A. 1945 Helminths from the American cotton-rat (*Sigmodon hispidus* Aun. Mag. Nat. Hist. **12**: 189–195.

———. 1936 The fauna of British India. Nematode 1. Ascaroidea and Strongyloidea. Taylor and Francis, London. 408 pp.

BELDING, D. L. 1936 Textbook of Clinical Parasitology. Appleton-Century-Crofts Inc., New York.

CLAPHAM, P. A. 1942 On identifying *Multiceps* spp. by measurements of the large hook. J. Helm. **20**: 31–40.

CLAUSSEN, L. 1933 Ueber haüfig bei der Biberratte vorkommende Darmparasiten. Deutsche Tierärztl. Wschr. **41**: 599–601.

COUTELEN, F., CALLOT, AND DESPORTES, C. 1939 Receptivite de l'ecureiul (*Sciurus vulgaris*) et du ragondin (*Myocastor coypus*)a l'echinococcose secondaire experimentale. Ann. Parasit. **17**: 162–166.

DAWES, B. 1946 The Trematoda. Cambridge University Press, London.

DIKMANS, G. 1937 A note on the members of the nematode genus *Trichostrongylus* occurring in rodents and lagomorphs, with descriptions of two new species. J. Wash. Acad. Sci. **27**: 203–209.

ECKMANN, F. 1932 Uber zwei neue Trematoden der Gattung *Aspidogaster*. Z. Parasitenk. **4**: 395–399.

ENIGK, K. 1933 Einige Nematoden aus der Nutria. Pelztierzucht, Leipzig **6**: 121.

ENIGK, K. 1950c Zur Epidemiologie des Strongyloidesbefalles des Sumpfbibers. Oesterr. Kleinterzuchter **23–24**: 874–875.

ERHARDOVA, B. 1958b Parasitičti červi hlodavcu Československa (Parasitische Wurmer der Nagetiere in der Tschechoslowakei) Česk. Parasitol. **5**: 27–103.

ENSMINGER, A. B. 1955 The economic status of nutria in Louisiana. Rept. to Fur and Refuge Div., La. Wildl. Fish Comm.

FREUND, L. 1930 Die Parasitien, Parasitaren und sonstigen Krankheiten der Pelztiere. Hanover.

GEBAUER, O. 1932 *Böhmiella perichitinea* n. sp. ein neuer trichostrongylide (Nematoda) der Nutria Ztschr. Parasitenk. **4**: 730–736.

GMEINER, F. 1931 Uber gehauftes Auftreten von Paratyphusfallen bein Nutria (Sumpfibiber, *Myocastor coypus*). Wiener Tierarztl. Monatsch. **18**: 140–152.

GRIEDER, H. 1937 Seltene Nutria Parasiten. Schweiz. Arch. Tierh. **79**: 520–525; 577.

———. 1938 Filaridae, Filariosis und Microfilariosis bein verschiedenen Saugetiere. Schweiz. Arch. Tierh. **80**: 185–490.

HALL, M. C. 1916 Nematode parasites of mammals of the orders Rodentia, Lagomorpha and Hyracoidea. Proc. U.S. Natl. Mus. **50**: 1–258.

———.1919 The adult taenoid cestodes of dogs and cats and of related carnivores in North America. Proc. U.S. Natl. Mus. **55**: 1–94.

HENRY, A. C. L. 1931 Les parasites et les maladies parasitaire du ragondin. Bull. Soc. Natl. Acclimat. France **78**: 421–447.

HEIDEGGER, E. 1931 Einige Peltztierparasiten in Wort und Bild. Deutsche Peltztierzucht **6**: 467–477.

HOHNER, L. 1954 *Cysticercus talpae* and *Cysticercus taeniaeformis* beim Sumpfbiber. Vorlaufige Mitteilung. Arch. Ex. Vet. Med.

8: 512–516.

HÜLPHERS, G. 1933 De hos pälsdjuren vanligast förekommende inälvsparasiterna. Skand. Vet. Tijdskr. Uppsala **23**: 133–154.

HUGGHINS' E. J. 1951 A survey of the helminths and ectoparsites of roof and cotton rats in Brazos County, Texas. Am. Midl. Nat. **46**: 230–244.

LAHILLE, F. 1918 Nota sobre *Monostoma mutabile* y la classificacion general de los trematodes, Buenos Aires. Physis: Rev. Soc. Argent. Cien. Nat. **4**: 328–331.

LENT, H. AND DEFREITAS, T. 1936 Sobre o *Trichuris* da nutria. Ann. Acad. Brasil. Sci. **8**: 319–323.

LORBER, E. 1932 Studie über die Verbreitung der Endoparasiten bei den Edelpelztieren in ČSR., ihre Diagnose und Pathogenetische Bedeutung. Klin. Spisy Vysoké Školy Zvěrolek., Brno. **9**: 155–173.

MEGGITT, F. J. 1924 The Cestodes of Mammals. London.

MILLER, C. C. AND HARKEMA, R. 1960 A new locality record for *Heterobilharzia americana* Price, 1929. J. Parasit. **46**: 206.

PACHECO, G. AND ARTIGAS, P. 1932 Parasitos do *Myocastor coipus*. Rev. Biol. e Hyg., S. Paulo **3**: 88.

PAGENSTECHER, H. A. 1877 Zur Naturgeschichte der Cestoden. Ztschr. Wiss. Zool. **30**: 171–193.

PETROV, A. M. AND SADYKHOV, I. A. 1959 (A new nematode *Longistriata* (*Brevispiculoides*) *myopotami* nov. sp. from the intestine of *Myopotamos coipys* in Azerbaidzhan). Dokl. Akad. Nauk Azerbaidzhan. SSR. **15**: 725–729.

PRICE, E. W. 1929 A synopsis of the trematode family Schistosomatidae with descriptions of new genera and species. Proc. U. S. Natl. Mus. **75**: 1–39.

————. 1943 A redescription of *Heterobilharzia americana* Price (Trematoda: Schistosomatidae). Proc. Helm. Soc. Wash. **10**: 85–86.

RAILLIET, A. AND MOUGUET, A. 1919 Cenure du Coypou. Bull. Soc. Cent. Med. Vet. **72**: 204–211.

REINISCH, M. 1940 Coprologic examination for animal parasites in the Zoological Garden in Budapest. Közlem. az Osszehas. Elet-és Kórtan Köréből **27**: 272–284.

RUBLI, H. 1936 Trichinose beim in Sumpfbiber, *Myocastor coypus* Mol. Schweiz. Arch. Tierh. **78**: 420–424.

SANDGROUND, J. H. 1925 Speciation and specificity in the nematode genus Strongyloides. J. Parasit. **12**: 59–80.

SCHACHER, J. F. 1955 Final report on parasites of Louisiana Mammals with emphasis on nutria (*Myopotamus coypus*) and otter (*Lutra canadensis* spp.). Submitted to the Fur and Refuge Division, Louisiana Wildl. and Fish. Comm.

SCHMID, G. 1933 Ergebnisse bei Peltztieruntersuchunger in Jahre 1932. Deutsche Pelztierzuchter, Munchen **8**: 307–311.

SCHÖNENBERGER, A. 1933 Magen- und Darmerkrankunger der Biberratte (*Myocastor coypus* Mol.) infolge Zooparasiteninvasion und deren Bekamfung. Inaug.-Diss. Vet. Med. Fak., Univ. Zurick.

SCHULZ, R. E. AND LANDA, D. M. 1934 Parasitische Wuermer der grossen Rennmaus—*Rhombomys opimus*. Licht. Rev. Microb., Epidemi and Parasit. **13**: 305–315.

SHUL'TS, R. E. S. 1931 (Parasitic worms of rabbits and hares and the diseases caused by them.)

SEDLMEIER, H. 1931 Ueber einem neuen Fadwurm (*Filaria* bzw. *Microfilaria kitti*) beim sumpfbiber. Deut. Pelztierzucht., Munchen **10**: 272–273.

SPASSKII, A. A. 1951 (Anoplocephalata-cestodes of domestic and wild animals). Moskva. Osnovy testodologii **1**: 1–735.

SPINDLER, L. A. 1942 Internal Parasites of Swine *in* Keeping Livestock Healthy. U.S.D.A. Yearbook.

SPREHN, C. 1930 Die Parasiten der Nutria. Pelztierzucht, Leipzig **6**: 121.

————. 1932 Lehrbuch Helminthologie. Berlin.

SWIERSTRA, JANSEN AND BROEK 1959 Parasites of zoo-animals in the Netherlands. Survey of zoo-animals in the Netherlands, identified from 1948–1958 inclusive. Tijdschr. Diergeneesk. **84**: 1301–1305.

TRAVASSOS, L. 1937 Revisao da familia Trichostrongylidae Leiper, 1912. Monog. Inst. Osw. Cruz, Rio de Janeiro **1** (parts 1–2).

————, VOGELSANG, E. 1930 Pesquizas helminthologicas realisadas em Hamburg. II Sobre dois trematodeos parasitos de mammiferos Mem. Inst. Osw. Cruz **23**: 169–171.

VOGELSANG, E. G. AND ESPIN, J. 1949 Dos nuevos huespedes para *Capillaria hepatica* (Bancroft, 1893) Travassos, 1915; nutria (*Myopotamus coypus*) y. el raton mochilero (*Adodon venezuelensis*). Rev. Med. Vet. y Parasit., Caracas **8**: 73–78

WARD, H. B. AND HOPKINS, S. H. 1931 A new North American aspidogastrid, *Lophotaspis interiora*. J. Parasit. **18**: 69–78.

WOLFFHÜGEL, K. 1916 El carpinchio (*Hydyrochoerus capybara*) huesped de *Fasciola hepatica*. Rev. Med. Vet. Montevideo, 1–227.

————. 1919 Servicio sanitario de la Inspeccion veterinaria mataderos liniers. Estadistica de equinococcosis de los animales faenados los en mataderos le liniers. Buenos Aires (1). Estadistica de distomatosis (*Fasciola hepatica* L.). Rev. Med. Vet. Montevideo **3**: 704.

————. 1920 Die Parasiten de Haustiere in Sudamerika, besonders in den La Platastaaten, Festochr. Feier 60. Geburst. Friedrich Zschokke **29**.

————. 1951 Nutria (*Myocastor coypus*) als Wirt von Echinococcus. Berl. w. München Tierärztl. Wchnschr. **4**: 78–79.

YAMAGUTI, S. 1958 Systema Helminthum: Digenetic Trematodes. **1** (parts 1–2): 1–1575.

ZAJIČEK, D. 1955 Parasitární invase w maladých nutrií. Sborn. Vysoké Zemědělske a Lesmické Fak Brne, Řada B. Spis. Fak Vet. **1**: 29–38.

Explanation Of Plates

(All figures diagramatic and drawn with aid of camera lucida or microprojector)

Plate I

Figure 1. *Heterobilharzia* sp. (male, ventral view).
Figure 2. *Heterobilharzia* sp. (female).
Figure 3. Unidentified schistosome (male).
Figure 4. *Echinostoma revolutum* (ventral view).
Figure 5. *E. revolutum* (anterior end).
Figure 6. *Psilostomum* sp. (Ventral view).
Figure 7. Unidentified aspidogastrid (ventral view).
Figure 8. *Neoechinorhynchus* sp. (juvenile).
Figure 9. *Neoechinorhynchus* sp. (anterior end).

Plate II

Figure 10. *Longistriata maldonadoi* (anterior end).
Figure 11. *L. maldonadoi* (male, posterior end).
Figure 12. *L. maldonadoi* (female, posterior end).
Figure 13. *Trichostrongylus sigmodontis* (anterior end).
Figure 14. *T. sigmodontis* (female, posterior end).
Figure 15. *T. sigmodontis* (female, ovejector region).
Figure 16. *T. sigmodontis* (male, posterior end).
Figure 17. *Strongyloides* sp. (anterior end).
Figure 18. *Strongyloides* sp. (female, ovejector region).
Figure 19. *Strongyloides* sp. (female, posterior end).

Plate III

Figures 20–23. *Trichuris myocastoris* (male, posterior end showing variations in shape of spicular sheath).

PLATE I

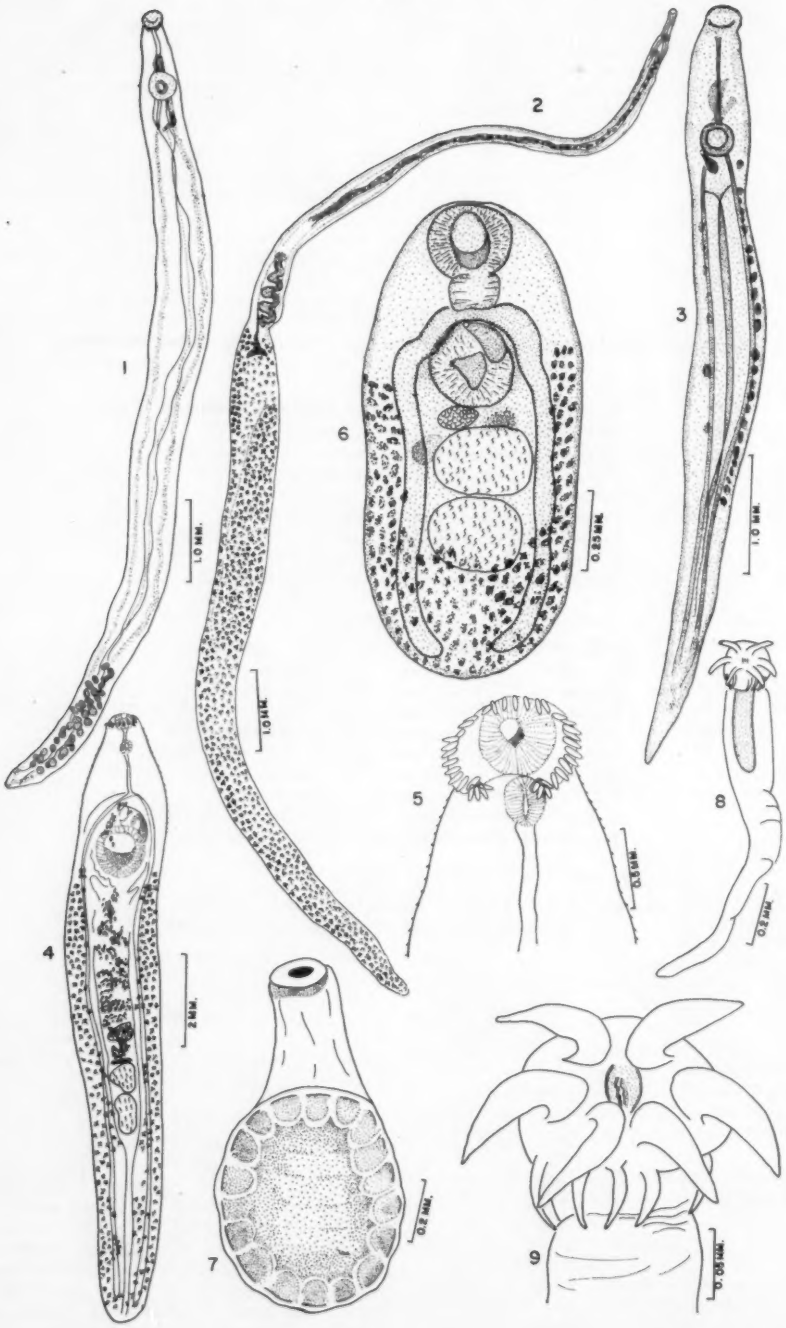


PLATE II

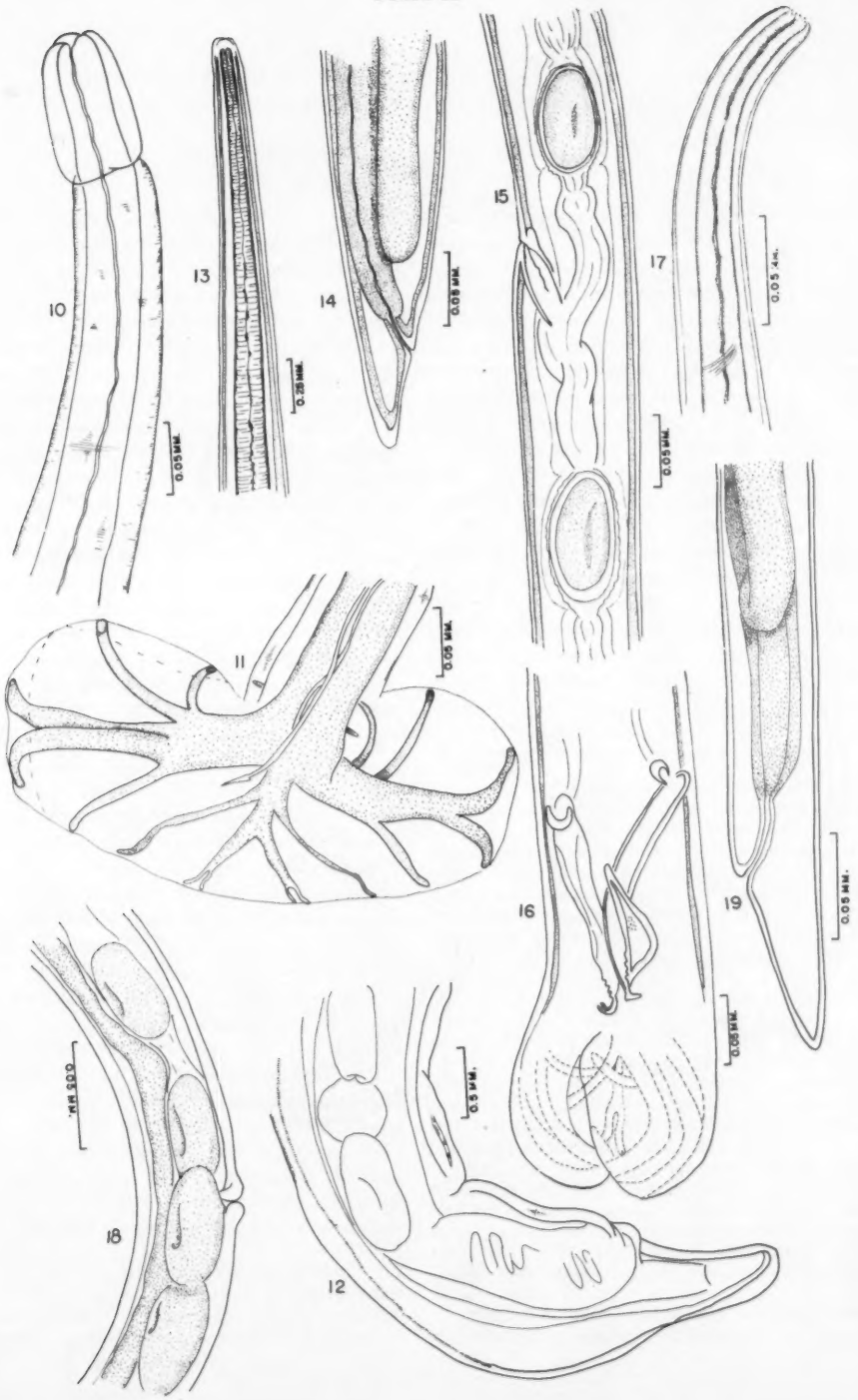
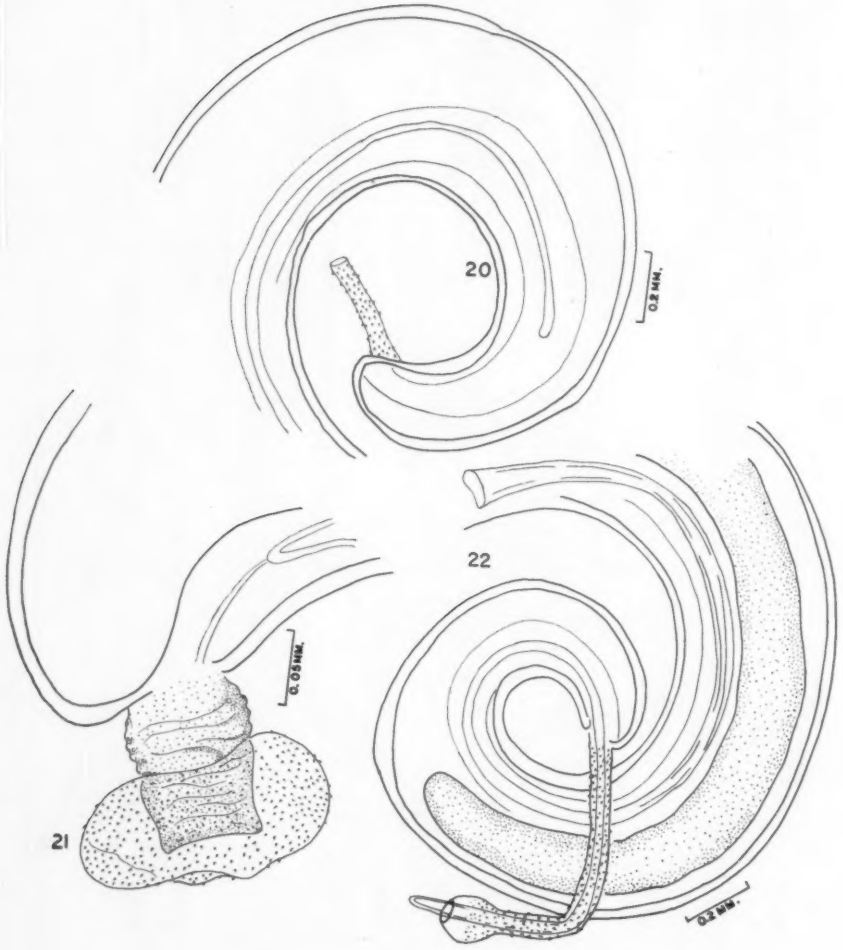

10
0.05 MM.
13
0.25 MM.
14
0.05 MM.
15
0.05 MM.
17
0.05 MM.
11
0.05 MM.
16
0.05 MM.
19
0.05 MM.
18
0.5 MM.
12
0.5 MM.

PLATE III



ENDOPARASITISM IN ISOLATED POPULATIONS OF RODENTS OF THE LAKE BONNEVILLE BASIN, UTAH*

JOHN C. FRANDSEN AND ALBERT W. GRUNDMANN
Department of Zoology and Entomology, University of Utah

The changing climate and physiognomy of the Lake Bonneville Basin during Pleistocene time have produced a mosaic of varyingly isolated gene pools among the rodents inhabiting that area. The presence within the basin of numerous recognized subspecific populations of several of the species of rodents attests to this fact. Populations of the southern pocket gopher, *Thomomys umbrinus*, have with particular frequency evolved to the degree where they are now recognized as constituting separate subspecies (Durrant, 1952). Although it is impossible to know with any degree of accuracy the length of time which has been required in a given case for daughter populations to become subspecifically distinct, the maximum time available for such evolution could not, with possibly one or two exceptions, exceed the length of time comprising the post-pluvial period (vide Antevs, 1948), because most of the areas now inhabited by the mammals under consideration here were submerged during the last pluvial period.

There is much controversy at present concerning the absolute length of post-pluvial time. Antevs (1948) dates the beginning of the first of his three intra-post-pluvial periods at 9,000 B.P. (before present). Post-pluvial time, however, may well be much longer than this (Eardley and Gvosdetsky, in press). In post-pluvial times, Lake Bonneville receded, and the formerly inundated area became habitable for rodents. The region then became much warmer; more arid and areas of high relief and of oasis became islands in a sea of desert. Rodents requiring nearby sources of drinking water and the edaphic and vegetational conditions associated with this water, or with high and well-drained localities, became restricted to such areas and isolated from others of their kind by arid uninhabitable wastes. The flow of genes between these populations was restricted in direct proportion to the vagility of the particular rodents concerned, to the width of the uninhabitable regions, and to the width of the applicable ecological tolerances of the rodents. The speed with which the frequency of occurrence of given genes in such isolated populations of the same species of rodent would change would be influenced in some cases at least by genetic drift.

In the summer of 1957, a study was undertaken to determine which visceral helminths and haemoprotista parasitize members of some of the species of native rodents of the Lake Bonneville Basin and to note any morphological divergences between populations of the same species parasitizing different kinds of hosts. During the subsequent 2 years, rodents were trapped alive at 46 widely separated trapsites situated at different elevations throughout the basin.

MATERIALS AND METHODS

In so far as time permitted, necropsies were conducted in the field. Blood for smears was drawn by means of cardiac puncture. These smears were stained with Giemsa's stain. Tissue impressions obtained on necropsy of *Neotoma* (vide infra) were stained with Giemsa's stain also. In some instances, these impressions were treated with a saturated solution of picric acid in 60 percent ethanol to remove pigment and other extraneous material (vide Boyd, 1949).

Living trematodes and adult cestodes were first placed in a vial of a hypnotic solution consisting of 2 g of trichlor-tertiary-butyl alcohol in 0.5 liter of 0.85 percent solution of sodium chloride (Hargis, 1953). Fixation was in hot formal-acetic-alcohol. Regressive staining was practiced with half-oxidized (vide Baker and Jordan, 1953) Delafield's or Ehrlich's iron-haematoxylin.

Encysted larval cestodes were either freed from their cysts with dissection needles or digested free by use of an aqueous solution of 1 percent pepsin and 0.7 percent hydrochloric acid (Belding, 1952, p. 968). Freed larvae with invaginated scolices were treated according to the technique of Edgar (1941).

Nematodes were killed by slowly heating them in saline, by the technique of Bailenger and Neuzil

Received for publication October 3, 1960.

* This study was supported by grants from the National Science Foundation (G-5280) and the University of Utah Research Committee.

(1953), or by placing them in boiling 70 percent ethanol. Worms killed by means other than immersion in ethanol were fixed in a solution consisting of 14 parts commercial formalin, 1 part glacial acetic acid, and 85 parts distilled water. This solution was recommended by Mr. G. Thorne of the University of Wisconsin (in verbis). The nematodes were cleared in either Amann's lactophenol or chlorolactophenol (for formulae see Langeron, 1949). They were examined in temporary mounts made of the clearant and then were rinsed in alkaline 70 percent ethanol and placed in neutral 70 percent ethanol for storage. Views en face were prepared according to the glycerin gel technique of Cobb as modified by Anderson (1958).

Acanthocephalans were relaxed, killed, and fixed according to the technique of Van Cleave (1953). They were stained with Van Cleave's combination haematoxylin and cleared in methyl salicylate.

RESULTS

In the following list, the number of hosts infected per number of hosts examined is given in parentheses. There is some difference in the total number of hosts of a given subspecies examined for haemoprotista and helminths due to the fact that hosts which had been dead for some considerable time prior to examination did not yield satisfactory blood smears. In the case of the anoplocephalid cestodes, the incidence of infection was low but undetermined due to mixed infections with large numbers of the tapeworms.

Parasite-Host List

Parasite	Host
PROTISTA	
Grahamella Brumpt, 1911	*Eutamias minimus pictus* (Allen, 1890) (2 :30)
	Microtus montanus nanus (Merriam, 1891) (1 :3)
	Perognathus formosus incolatus Hall, 1941 (3 :6)
	P. parvus olivaceous Merriam, 1889 (4 :25)
	Peromyscus maniculatus rufinus (Merriam, 1890) (3 :40), *P. m. sonoriensis* (Le Conte, 1853) (6 :200)
	Thomomys talpoides wasatchensis Durrant, 1946 (1 :75)
	T. umbrinus centralis Hall, 1930 (2 :18), *T. u. stransburyi* Durrant, 1946 (1 :25)
Trypanosoma Gruby, 1843	*Eutamias minimus pictus* (1 :20)
	Peromyscus maniculatus rufinus (1 :40), *P. m. sonoriensis* (5 :200)
NEMATODA	
Ascaridae	
Ascaris laevis Leidy, 1856	*Thomomys talpoides wasatchensis* (4 :108)
	T. umbrinus albicaudatus Hall, 1930 (12 :79)
Heligmosomidae	
Brevistriata skrjabini (Schultz and Lublmov, 1932)	*Peromyscus maniculatus sonoriensis* (1 :280)
	Thomomys talpoides wasatchensis (5 :108)
Nippostrongylus muris (Yokogawa, 1920)	*Peromyscus maniculatus sonoriensis* (3 :280)
Oxyuridae	
Syphacia peromysci Harkema, 1936	*Peromyscus maniculatus sonoriensis* (40 :280), *P. m. rufinus* (12 :58)
	Reithrodontomys megalotis megalotis (Baird, 1858) (4 :21)
Syphatineria eutamii (Tiner, 1948)	*Eutamias amoenus amoenus* (Allen, 1890) (1 :4)
	E. dorsalis utahensis Merriam, 1897 (1 :2)
	E. minimus consobrinus (Allen, 1890), (3 :12), *E. m. pictus* (4 :32)
Wellcomia evoluta (Linstow, 1899)	*Marmota flaviventer nosophora* Howell, 1914 (1 :3)
Subuluridae	
Gongylonema sp. (new)	*Peromyscus maniculatus sonoriensis* (1 :280)
Protospirura numidica Seurat, 1914	*Peromyscus maniculatus sonoriensis* (25 :280)
	P. truei nevadensis Hall and Hoffmerster, 1940 (1 :4)
	Reithrodontomys megalotis megalotis (1 :20)
Rictularia coloradensis Hall, 1916	*Peromyscus crinitus pergracilis* Goldman, 1939 (1 :3)
	P. maniculatus sonoriensis (17 :280)
	Perognathus parvus olivaceus (1 :30)
Rictularia sp. (probably *R. coloradensis*)	*Eutamias minimus pictus* (2 :32)
	E. amoenus amoenus (1 :4)
Strongylidae	
Ransomus rodentorum Hall, 1916	*Thomomys talpoides gracilis* Durrant, 1939 (19 :41), *T. t. wasatchensis* (8 :108)
	T. umbrinus albicaudatus (1 :79), *T. u. stansburyi* (3 :28)
Spiruridae	
Heteroxynema cucullatum Hall, 1916	*Eutamias amoenus amoenus* (1 :4)
	E. dorsalis utahensis (1 :2)
	E. minimus pictus (10 :32)
	E. umbrinus umbrinus (Allen, 1890) (4 :7)
Trichuridae	
Capillaria bonnevillei Grundmann and Frandsen, 1960	*Dipodomys ordii marshalli* Goldman, 1937 (5 :23)
Trichuris fossor Hall, 1916	*Thomomys talpoides gracilis* (8 :41), *T. t. moorei* Goldman, 1938 (1 :5), *T. t. wasatchensis* (45 :108)
	T. umbrinus albicaudatus (22 :79), *T. u. aureiventris* Hall, 1930 (1 :5), *T. u. centralis* (5 :20)
Trichuris sp. (new)	*Peromyscus maniculatus sonoriensis* (4 :280)
CESTODA	
Anoplocephalidae	
Cittotaenia praecoquis (Stiles, 1895), Stiles and Hassall, 1896	*Thomomys talpoides*
Paranoplocephala infrequens (Douthitt, 1915)	*Thomomys talpoides*

Paranoplocephala variabilis (Douthitt, 1915)	*Thomomys talpoides*
Catenotaeniidae	
Catenotaenia linsdalei McIntosh, 1941	*Perognathus formosus incolatus* (1 :6) *Peromyscus maniculatus sonoriensis* (10 :280)
Dilepidinae	
Paruterina candelabraria (Goeze, 1781), Fuhrmann, 1906 (plerocercoids)	*Peromyscus maniculatus sonoriensis* (1 :280)
	P. truei nevadensis (1 :4) *Thomomys umbrinus aureiventris* (1 :5)
Hymenolepididae	
Hymenolepis citelli McLeod, 1933	*Peromyscus maniculatus sonoriensis* (4 :280) *Thomomys talpoides wasatchensis* (8 :108) *T. umbrinus albicaudatus* (1 :79), *T. u. stansburyi* (1 :28)
Hymenolepis horrida (von Linstow, 1901)	*Thomomys talpoides wasatchensis* (7 :108)
Mesocestoididae	
Mesocestoides carnivoricolus Grundmann, 1956 (plerocercoids)	*Peromyscus maniculatus sonoriensis* (2 :280)
	P. crinitus pergracilis (1 :3)
Taeniidae	
Cladotaenia cerci Yamaguti, 1935 (plerocercoids)	*Peromyscus maniculatus sonoriensis* (1 :280)
TREMATODA	
Brachylaimidae	
Brachylaime micrati Kruidenier and Gallicchio, 1959	*Peromyscus maniculatus sonoriensis* (3 :280)
ACANTHOCEPHALA	
Moniliformidae	
Moniliformis clarki (Ward, 1917)	*Peromyscus maniculatus sonoriensis* (4 :280) *Thomomys umbrinus stansburyi* (1 :28)

DISCUSSION

Because only dry blood smears, prepared at the time of death of the rodents, were available for study, the identification of the haemoprotistans found in these animals was with one notable exception (vide infra) based only on dry, fixed specimens. The identification of *Grahamella* is, consequently, based only on the appearance of the intraerythrocytic bodies found in the blood smears; no data are available concerning cultural characteristics. In view of the findings of Davis (1952) it was not believed advisable to assign the trypanosomes to species. They belong to the *lewisi* group.

The examination of a large number of males of the strongyloid nematode *Ransomus rodentorum* has shown that there is great variability in the degree of separation of the branches of the dorsal ray of the bursa. In his description of the species, Hall (1916) describes the dorsal ray as being divided into two branches, each in turn divided once. The present study has shown that this latter division may not occur; when it does, it may be at a widely variable distance from the distal end of the dorsal ray.

Anoplocephalid cestodes were found only in northern pocket gophers occuring in the Wasatch and Raft River Mountains. Cestodes of this family, of yet uncertain genus, were also recovered from southern pocket gophers taken in the Oquirrh Mountains. Anoplocephalids apparently have been unable to cross the desert regions of the floor of the basin. Douthitt (1915) noted that the anoplocephalids of rodents appear generally restricted to hosts living in comparatively moist montane habitats; this would appear to be the case in the Lake Bonneville Basin. Where these cestodes were found, many pocket gophers were parasitized; there were as many as 40 mature cestodes per infected pocket gopher.

No consistent morphological differences were found between individuals of the same species of parasite found in different species or subspecies of hosts. In general, it is quite possible that failure to find given parasites in areas where suitable hosts occur may be explained on the basis of insufficient size of sample. It is unfortunate that time did not permit conducting a survey of intermediate hosts. It is believed that in the Lake Bonneville Basin the patterns of distribution of *Ransomus rodentorum*, *Trichuris fossor*, *Capillaria bonnevillei*, and the species of *Trichuris* and *Gongylonema* found in *Peromyscus maniculatus* are of historical significance. An analysis of the distribution of *Ransomus rodentorum* and *Trichuris fossor* has been presented elsewhere (Frandsen and Grundmann, in press).

The species of *Trichuris* and *Gongylonema* represented in *Peromyscus maniculatus* are new; descriptions are in press. These two species and *Capillaria bonnevillei*, as well as an apparently new species of *Capillaria* found in *Peromyscus maniculatus*, are seemingly confined to Stansbury Island in the Great Salt Lake. The incidence of parasitism with these helminths is low and the number of worms per host is also low. Genetic drift has quite probably played an important role in the evolution of these species on

Stansbury Island. Three subspecies of cricetid mice and one subspecies of pocket gopher are endemic on this island, indicating that some of the populations of rodents found there have been isolated from populations on the mainland for a rather long period of time.

In addition to the parasites cited in the preceding list, an unidentified haemoparasite was observed within the erythrocytes of representatives of *Eutamias minimus pictus*, *E. minimus pictus* × *E. minimus consobrinus* intergrades, and *Neotoma lepida lepida* Thomas, 1893. The parasite was first encountered in smears of blood from *E. minimus pictus* taken in the Raft River Mountains of extreme northwestern Utah. In this blood, the parasite bore a striking resemblance to the signet ring stage of trophozoites of *Plasmodium*. Parasites with both one and two masses of chromatin, staining red with the Romanovsky stains, were found. Multiple infections of a single erythrocyte were not uncommon, and these multiple infections, combined with the rather common occurrence of signet rings with two chromatin dots, were reminiscent of *Plasmodium falciparum*. Infected erythrocytes were not enlarged, and no pigment was found therein. Fewer than 1 percent of the erythrocytes in average positive blood smears was infected. Parasites with similar morphology and similar in number were found later in the blood of intergrades of *E. minimus pictus* × *E. minimus consobrinus* taken in the Oquirrh Mountains southwest of Salt Lake City. In neither case did the infected chipmunks exhibit symptoms of disease during the short time they were held prior to necropsy. In a previous report (Frandsen and Grundmann, 1959), these parasites were referred to the genus *Plasmodium*. This identification cannot be confirmed at present.

Examination of smears of blood of *Neotoma lepida lepida* taken in the Deep Creek Mountains of extreme west-central Utah also revealed the presence of intraerythrocytic structures resembling the signet ring stage of *Plasmodium* trophozoites. Few rings with two chromatin dots were seen, and multiple infections of erythrocytes were rare. Living specimens of *Neotoma lepida* were captured later at the same trapsite for the purpose of obtaining a number of successive blood smears from them and of attempting transfer of infection via inoculation of infected blood. These animals were bled repeatedly for examination of blood during the autumn of 1959. The following stages of the blood parasite in question were found during the course of these repeated examinations:

1. Stages resembling the signet ring stage of trophozoites of *Plasmodium* (fig. 1). These rings

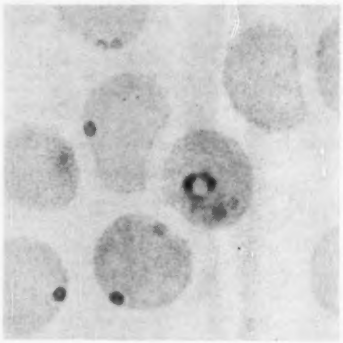


FIGURE 1. Photomicrograph of blood of *Neotoma lepida* showing signet-ring stage of parasite within erythrocyte. Giemsa's stain.

were overwhelmingly one-chromatin-dot rings. Very few erythrocytes contained more than one parasite.

2. Stages resembling somewhat older trophozoites of *Plasmodium*. These exhibited the polymorphism which one would expect to see with amoeboid organisms, including trophozoites of *Plasmodium*.

3. Occasionally stages were seen resembling the *tenue* form (or "aestivo-autumnal 'slender forms'" of Wilcox, 1950) of trophozoites of *Plasmodium falciparum*.

4. A spleen impression prepared at necropsy of an infected animal which died from an overdose of anesthetic contained intraerythrocytic inclusions appearing as five solid red bodies of equal size, as stained with Giemsa's stain, in a mass of blue (cytoplasm ?) (fig. 2). This impression had previously been treated to remove malarial pigment and other extraneous material. Other impressions of liver, spleen, brain, and kidney have not contained these intraerythrocytic inclusions.

The proportion of infected erythrocytes in the circulating blood of these wood rats was usually low to very low, i. e., from 1:100 to 1:1000 to 1 or 2 per thin smear. The level of parasitemia in blood of a given *Neotoma* varied greatly during the course of time.

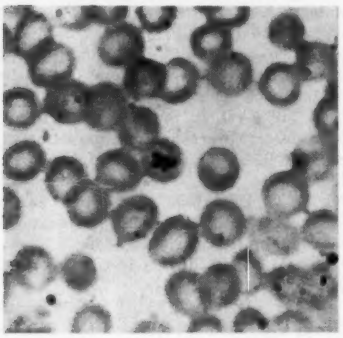


FIGURE 2. Photomicrograph of spleen impression of *Neotoma lepida* showing possible division stage of parasite within erythrocyte. Giemsa staining after previous treatment with picric acid-ethanol.

Six more infected woodrats were trapped on the eastern slopes of the Deep Creek Mountains during December 1959. The level of parasitemia was high, 10 percent or more of the erythrocytes being parasitized. All of these animals died within 30 days after being brought to the laboratory, and several of them showed symptoms of cachexia prior to death. On account of postmortem changes, the tissues were not suitable for pathological and parasitological examination.

Numerous attempts have been made to transfer the infection to uninfected woodrats via intramuscular and intraperitoneal inoculation of infected citrated blood. All of these attempts have met with failure.

At present it is impossible to decide whether to assign this parasite to the superfamily Babesioidea Poisson, 1953, or the family Plasmodiidae. In order to assign the parasite to the genus *Plasmodium,* it would be necessary to find other stages, including sexual stages, and to find pigment in the infected erythrocytes during some stage of the life cycle of the parasite. *Plasmodium*-like characters of the parasite as known at present include the finding only of stages resembling *Plasmodium* in the erythrocytes of heart and peripheral blood. If the inclusions found in erythrocytes in the spleen impression were stages of the parasite, they would resemble the schizonts of certain described species of avian *Plasmodium* which produce a relatively small number of merozoites with large masses of chromatin. If this parasite were a species of *Plasmodium,* however, the failure to find either miero- or macrogametocytes would be much more difficult to explain than the absence of asexual stages more advanced than signet-ring trophozoites.

The absence of malarial pigment and of *Plasmodium*-like gametocytes and advanced asexual stages from the peripheral blood, however, suggests that this parasite may be a species of *Babesia* Starcovici, 1893. Our knowledge of the species of this genus found in wild animals, however, is probably very inadequate, and present concepts of the genus may be modified in the future. Perhaps the inclusions are not of a parasitic nature.

ACKNOWLEDGMENTS

The authors wish to acknowledge the very kind advice and interest of Mrs. M. B. Chitwood and Mr. Allen McIntosh of the U.S.D.A. Agricultural Research Center, Dr. G. Robert Coatney of the National Institutes of Health, Dr. Reino S. Freeman of Ontario Research Institute, and Dr. Marietta Voge of the University of California at Los Angeles.

SUMMARY

A survey has been conducted of the haemoprotista and visceral helminths parasitizing rodents occurring, in many cases, as isolated subspecific populations. A list of the species of parasites and hosts is included. It is concluded that little morphological divergence has occurred between members of isolated populations of parasites during a time sufficient for subspecific divergence of their hosts. Morphological variation in the nematode *Ransomus rodentorum* and the status of a haemoparasite of uncertain affinities are discussed.

LITERATURE CITED

ANDERSON, R. C. 1958 Methode pour l'examen des Nematodes en vue apicale. Ann. Parasit. **33**: 171–172.

ANTEVS, E. 1948 The Great Basin, with emphasis on glacial and post glacial times. III. Climatic changes and pre-white man. Bull. Univ. Utah **38**: 178–191.

BAILENGER, J. AND NEUZIL, E. 1953 Nouvelles Techniques d'examen des Helminthes: fixation; coloration; montage. Ann. Parasit. **28**: 392–398.

BAKER, J. R. AND JORDAN, B. M. 1953 Miscellaneous contributions to microtechnique. Quar. J. Micr. Sci. **94**: 237–242.

BELDING, D. L. 1952 Textbook of Clinical Parasitology. 2d ed. Appleton-Century-Crofts, Inc., N.Y.

BOYD, M. F. (Editor) 1949 Malariology. Saunders and Co., Philadelphia.

DAVIS, B. S. 1952 Studies on the trypanosomes of some California mammals. Univ. Calif. Publ. Zool. **57**: 145–250.

DOUTHITT, H. 1915 Studies on the cestode family Anoplocephalidae. Ill. Biol. Monog. **1**: 353–446.

DURRANT, S. D. 1952 Mammals of Utah, taxonomy and distribution. Univ. Kans. Publ., Mus. Nat. Hist. **6**: 1–549.

EARDLEY, A. J. AND GVOSDETSKY, V. Pleistocene (Saltair) core from Great Salt Lake. Bull. Geol. Soc. Am. In press.

EDGAR, S. A. 1941 Use of bile salts for the evagination of tapeworm cysts. Tr. Am. Micr. Soc. **60**: 121–128.

FRANDSEN, J. C. AND GRUNDMANN, A. W. 1959 Preliminary report on the haemoparasites of rodents of Lake Bonneville Basin, Utah. J. Parasit. **45** (Supp.): 33.

——— AND ——— The history of some subspecies of pocket gophers as told by their monoxenous nematodes. Proc. Utah Acad. Sci., Arts, and Ltrs. In press.

HALL, M. C. 1916 Nematode parasites of mammals of the orders Rodentia, Lagomorpha, and Hyracoidea. Proc. U. S. Natl. Mus. **50**: 1–258.

HARGIS, W. J., JR. 1953 Chloretone as a trematode relaxer, and its use in mass-collecting techniques. J. Parasit. **39**: 224–225.

LANGERON, M. 1949 Precis de microscopie. 7th ed. Masson and Cie, Paris.

VAN CLEAVE, H. J. 1953 Acanthocephala of North American mammals. Ill. Biol. Monog. **23**: 1–179.

WILCOX, A. 1950 Manual for the Microscopical Diagnosis of Malaria in Man. 2d ed. Natl. Inst. Hlth. Bull. No. 180.

SUGGESTIONS TO PROSPECTIVE AUTHORS

III. THE RESEARCH NOTE.

For many years the Journal of Parasitology has published short papers under the heading "Research Note." The research note, as the name implies, should be short and it should report research. Further than that it is difficult to define, but there have always been certain editorial views as to its nature though, understandably, strict adherence to them has not been observed. There follows a short summary of editorial views concerning its scope.

First, it is to be emphasized that it should not constitute a so-called "preliminary report." The Journal does not have sufficient funds to support double publication; hence, other media should be utilized by those who have reasons for desiring to publish preliminary notes.

Nor should the practice of using it for reporting freakish phenomena, such as morphological anomalies or occurrence of the parasite in abnormal habitats, be encouraged, especially in cases where previous well-known records exist; e.g., *Ascaridia* in a hen's egg. It cannot be denied, however, that reporting of abnormalities which have some fundamental significance is justifiable, and striking anomalies not previously reported could deserve publication.

The typescript for the research note should conform exactly to the style adopted by the Journal. The heading RESEARCH NOTE in capital letters is centered at the top. Underneath is the title, also in capitals. The author's name and address appear at the end, separated by a dash. Bibliographic references appear only in the text; author's initials and titles of articles are omitted (see J. Parasit. **45**(6): 621–622). Suggested abbreviations for names of selected journals have been published (J. Parasit. **46**(3): 308, 316, 326). Publication of figures and tables with research notes is discouraged, though not forbidden.

Research notes are used principally in spaces on pages otherwise only partly filled, though occasionally a blank page is available. There is no guarantee that they will not be split when they exceed one printed page in length. Manuscripts which will require two or more pages when published should ordinarily be in the form of regular articles. Illustrations of the form employed for research notes are to be found in any recent number of the Journal.—ELERY R. BECKER, *Editor*.

PERIGLISCHURUS TRIAENOPSIS N. SP. PARASITIC ON *TRIAENOPS AFER* PETERS (ACARINA: MESOSTIGMATA: SPINTURNICIDAE)*

P. L. G. BENOIT**
Musée Royal de l'Afrique Centrale, Tervuren

Periglischrus triaenopsis n. sp.

Host: *Triaenops afer* Peters (Hipposideridae).

Locality: Tanga, Sigi River, Tanganyika Territory.

Collector: Dr. H. Hoogstraal (709, 747, 749).

Material: Holotype female and allotype male in U. S. National Museum, Washington, D. C. 16 female, 2 male paratypes, 2 nymphs. Paratypes in U. S. National Museum and Musée Royal de l' Afrique Centrale, Tervuren.

Female: Body length, excluding gnathosoma, 819 microns in holotype, 766 to 846 microns in paratypes. Venter: Sternal plate shaped as figured (fig. a). Three pairs of minute sternal setae all situated on the sternal plate. Genital plate very weakly sclerotized, crescent-shaped. Metasternal setae level with anterior border of genital plate. The venter presents three pairs of additional setae in divergent rows. Anal setae slightly longer than other ventral setae. Anus with two sclerotized lobes and a narrow crescent-shaped formation posterior to them. Posterior part of venter shows a sclerotized area, with rugose sculpturing; this is subject to variation in form and size, but in most females is as in figure a. This area is visible in engorging females, but appears to be absent in all undistended females. In this respect, the present species confirms the presence or absence of the differently shaped sclerotized anal areas in the females of *P. moucheti* as described by Till (1958). Dorsum: Dorsal platelets have not been observed in any specimens, possibly having been rendered translucent by treatment with caustic potash 10 percent solution. Peritreme dorsal, situated between coxae III and IV. Eleven pairs of minute pores (the remains of setae?) arranged as shown in figure b, with one pair of setae situated opposite coxae IV. Legs: Median dorsal hair of femur I, 71 microns long, slightly shorter than width of femur; width of femur, 76 microns. Chaetotaxy of tarsus I as shown in figure d.

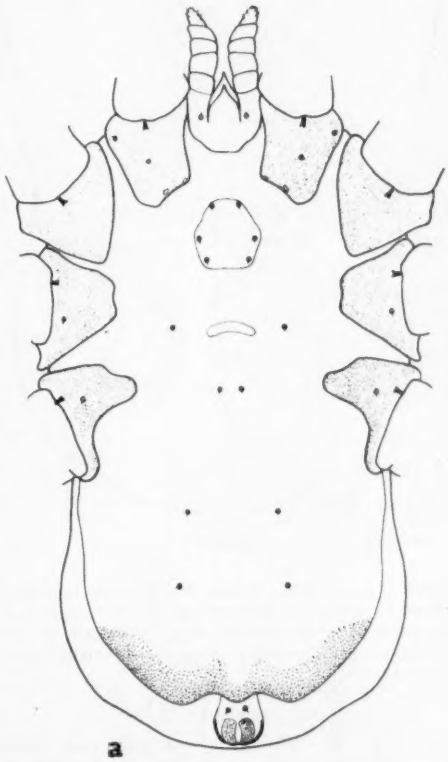


FIGURE a. *Periglischrus triaenopsis* n. sp. female, venter.

Male: Body length, excluding gnathosoma, 385 to 395 microns. Greatest width 320 microns. Sternal plate laterally extended, with one pair of setae. Soft integument of venter with seven pairs of setae, including one pair of anal ones. Two long lobes occur lateral to the anus, extend beyond the posterior limits of the body, each with a long seta dorsally. Dorsum as in female. Legs: Median dorsal hair of femur I, 80 microns long; of corresponding genu, 63 microns long.

This species keys to *P. africanus* Zumpt in Till's (1958) key to the Ethiopian species of the genus, with which it agrees in having the same

Received for publication December 12, 1960.

* This report is a contribution from the Scientific Working Party on Ectoparasites sponsored by the U. S. Naval Medical Research Unit No. 3 and the East African Veterinary Research Organization, 1956.

** Consultant, Medical Zoology Department, U. S. National Museum and Musée Royal de l' Egypt.

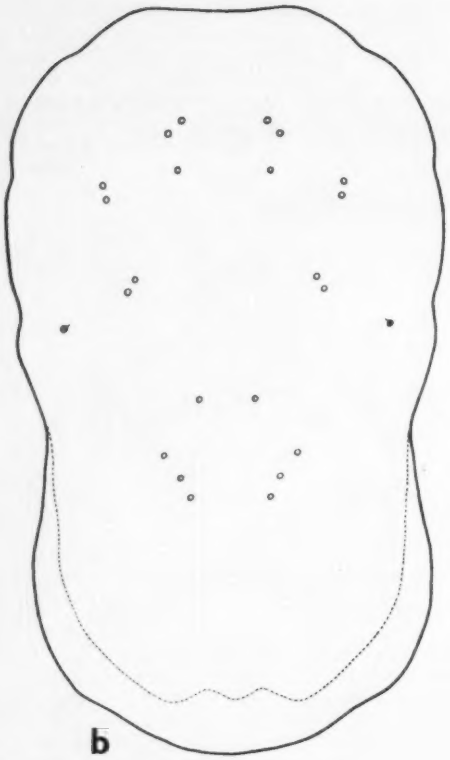


FIGURE b. Female, dorsum.

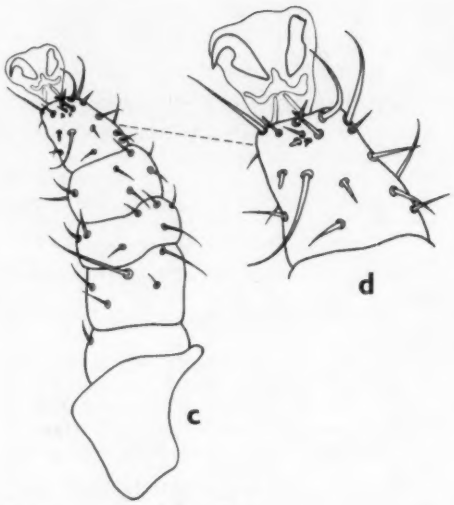


FIGURE c. Female, leg I.
FIGURE d. Tarsus I and claw.

general shape of the female sternal plate. It differs from *P. africanus*, however, in the female genital plate and of the dorsum, the chaetotaxy of the venter, and the presence of two long perianal lobes in the male.

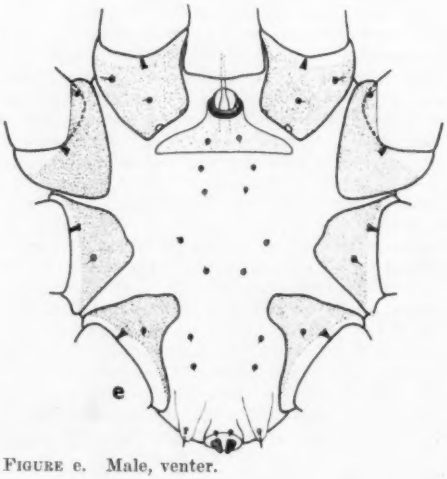


FIGURE e. Male, venter.

REFERENCE

TILL, W. M. 1958 Five new species of mites (Acarina: Laelaptidae and Spinturnicidae) parasitic on bats in the Ethiopian Region, with a key to the species of the genus *Periglischrus*. Revue Suisse Zool. 65: 241–258.

MORTALITY OF EASTERN BROOK TROUT CAUSED BY PLEROCERCOIDS (CESTODA: PSEUDOPHYLLIDEA: DIPHYLLOBOTHRIIDAE) IN THE HEART AND VISCERA

GLENN L. HOFFMAN AND C. E. DUNBAR

Bureau of Sport Fisheries and Wildlife, Eastern Fish Disease Laboratory, Leetown (P.O. Kearneysville), W. Va.

It is well known that migrating helminth larvae do a great amount of damage to the host. Often, however, the host is relatively large and the damage done by few larvae is not serious. When the host is very small the mechanical damage, at least, must be proportionately greater for each larva present. An unusual case of mortality of small brook trout (*Salvelinus fontinalis*) roughly 2 to 4 inches long was brought to our attention. Examination of the mortalities, as well as survivors, revealed relatively large diphyllobothrid larvae (fig. 1) in the chambers of the heart, liver, pericardial sac, and body cavity. Death of the fish was attributed to hemorrhaging caused by the migrating larvae. Some larvae appeared to be quiescent and were surrounded with sparse connective tissue and very little white cell infiltration. There was some tissue necrosis adjacent to some of the worms, and the livers were "fatty" with foam fat and sparse fat vacuoles. We do not believe that either of these conditions contributed to the mortalities. No other disease entities could be found. Proteocephalid larvae were present in the intestine, but there was no evidence that they contributed to the mortalities.

The epizootic has been well recorded by Mr. J. L. Jorgensen, Fisheries Manager, Canadian International Paper Co., Woodlands Division, Three Rivers, Quebec, Canada, and a summary of his 1958 report, "Growing Trout on Plankton at La Tuque Mill", is given here. This unique hatchery is located at La Tuque Mill, La Tuque, Quebec, which draws its water supply from Lake Wayagamack at the 50 ft-depth level. The filter reject water from this water supply is the exclusive water supply for the hatchery. It contains such large quantities of plankton, mostly copepods, that no other food is needed.

In 1956, 1000 fingerlings were successfully raised with little mortality. Fingerlings were

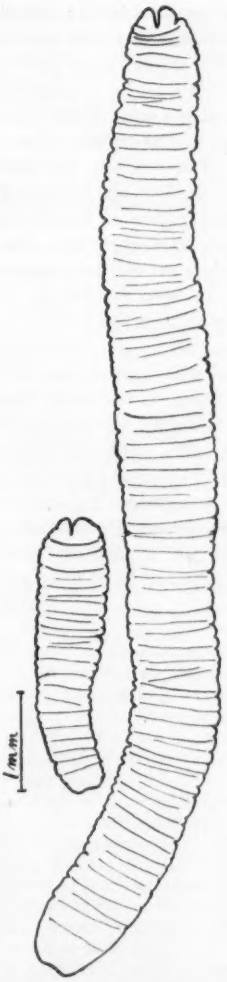


FIGURE 1. Largest and smallest diphyllobothrid plerocercoids found in the brook trout epizootic (drawn to scale with microprojection).

Received for publication December 9, 1960.

obtained in September and kept until November with over 50 percent weight gain. In 1957 Mr. Jorgensen started with eggs in January but lost 80 percent of the fish by November. Cause of death was determined by R. T. Reppert, biologist, to be due to the tapeworm larvae. Hemorrhage from parasitized hearts was visible in the intact fish as "red blotchy markings" anteroventral to the heart. A similar outbreak occurred in 1958.

Four larvae, fixed in situ with formalin and stained and mounted in Permount measured 2.9, 4, 9, and 10.6 mm in length and 0.28, 0.4, 0.42, and 1 mm, respectively, in greatest width. This larva is about the same width throughout most of its length and is transversely wrinkled, not segmented. The gonads are not developed. Bothria are shallow and connected by a deep dorsal-ventral groove or invagination over the anterior end, somewhat like *Schistocephalus,* but the strobila more nearly resembles *Diphyllobothrium.* In cross sections the thick cuticle, 5 to 10 microns thick, and the cuticular bristles can be seen. The thick cuticle is eosinophilic when stained with hematoxylin and eosin and is light blue when stained with Mallory's triple stain. This worm belongs in the family Diphyllobothriidae Lühe, 1910, but cannot be further identified until life history studies are made.

To our knowledge the only other North American record of diphyllobothrid larvae in the heart of fish is that of Moore, E. (1925, 1926). She reported an interesting epizootic of frost fish (*Coregonus quadrilateralis*) infected with a *Bothriocephalus* sp. Mortalities were caused by perforation of the auricle of the heart and adjacent veins. The specific identity of the worm could not be determined.

Dr. Marvin C. Meyer and Dr. Roth Vik (personal communication) occasionally found diphyllobothrid plerocercoids in the heart ventricles of the few adult salmonids available to them in Maine during the summer of 1960.

In Europe plerocercoids involving the heart of fish have been recorded by Vogel (1929), who found them on the heart and in the head of burbot, and Bergman (1923) also found them involving the heart.

We wish to thank Dr. R. A. Wardle, Winnipeg, Canada, for examining our slides and Dr. M. C. Meyer, University of Maine, for the literature references and other assistance.

REFERENCES

BERGMAN, A. V. 1923 Larver av *Triaenophorus robustus* Olsson och en Dibothriocephalus-art som orsak till massdöd bland unga laxfiskar. Skand. Vet.-Tidspr. **13**: 7–43.

MOORE, E. 1925 Diseases of Fish. 14 Ann. Rep. N. York State Conservation Dept. (1924), pp. 83–97.

———— 1926 Fish Diseases. 15 Ann. Rep. N. York State Conservation Dept. (1925), pp. 139–146.

VOGEL, H. 1929 Helminthologische Beobachtungen in Ostpreussen, insbesondere über *Dibothriocephalus latus* und *Opisthorchis felineus.* Deut. Med. Wchnschr. **55**: 1631–1633.

CARBOHYDRATE CONTENTS OF THE TAPEWORM *HYMENOLEPIS DIMINUTA* FROM NORMAL, BILELESS, AND STARVED RATS*

CHAUNCEY G. GOODCHILD
Department of Biology, Emory University, Atlanta 22, Georgia

Failure of *Hymenolepis diminuta* (Rudolphi, 1819) to thrive in the small intestine of Wistar strain rats surgically deprived of bile has been reported in previous studies (Goodchild, 1958, 1960). The present study was undertaken to ascertain whether the carbohydrate contents of tapeworms recovered in the earlier study (Goodchild, 1960) from normal, bileless, and starved hosts might give insight into the nature of the suppressive effect due to lack of bile.

MATERIALS AND METHODS

One 18-day-old cysticercoid of *H. diminuta* from infected flour beetles (*Tribolium confusum*) was fed by stomach tube to each of 56 Wistar strain rats (270 to 280 g and within 2 days of the same age). On the 20th day, when tapeworm eggs were appearing in the feces, the infected rats were divided into 2 experimental and 1 control groups and treated as follows: (1) 16 rats were taken off food and starved for the next 7 days; (2) an operation was performed on each of 25 rats to cannulate the bile duct to the caecum, according to the method given in a previous paper (Goodchild, 1958), and the rats were given ad libitum a standard laboratory food for the next 7 days; and (3) 15 unoperated rats remained on the normal laboratory diet for the next 7 days as a control group.

On the 27th day after infection each rat was sacrificed and the intestine examined for a tapeworm. When one was found its wet weight was recorded, the whole worm was placed in distilled water, homogenized, stored in the deep freeze, and later freeze-dried for dry weight determinations and subsequent chemical analysis for carbohydrate contents.

Of 15 freeze-dried *H. diminuta* recovered from 15 normal rats 9 were analyzed for total carbohydrate and glycogen. Nine tapeworms selected randomly from 11 recovered from 17 surviving bileless hosts and 9 others selected randomly from 11 recovered from the 16 starved hosts were analyzed for total carbohydrate and/or glycogen based upon the dry weight of the whole worm.

Determinations of total carbohydrate in these dried specimens were performed as follows: Samples (5 to 10 mg) of tissue were digested for 45 minutes with 2 ml 30 percent KOH in a water bath at 100 C and were then diluted to 50 ml with distilled water. Anthrone determinations were made on five 1-ml portions of each diluted digest. Total carbohydrate was calculated by comparison with anthrone tests made simultaneously on a series of glycogen standards.

Glycogen determinations in dried specimens were performed following, in general, the procedure of Harrow et al (1960): Samples (7 to 12 mg) of tissue were digested for 45 minutes with 2 ml 30 percent KOH in a water bath at 100 C and then cooled in water. Two drops of 10 percent $ZnSO_4$ and 4 ml of 95 percent ethanol were added to each digest; the mixture was stirred, heated to boiling in a water bath, cooled, and centrifuged. The supernatant was discarded and the precipitated glycogen dissolved in 2 ml water and reprecipitated with 4 ml ethanol. After a second boiling and cooling, the mixture was centrifuged and the supernatant discarded. The tube containing the precipitated glycogen was heated in a water bath to evaporate the remaining ethanol. The glycogen was dissolved in water and diluted to 50 ml if the worm was from a normal host, to 25 ml if the worm was from a bileless host, or to 10 ml if the worm was from a starved host. Anthrone determinations were made on five 1-ml portions of each sample of dissolved glycogen. The amount of glycogen was again calculated from anthrone tests made concurrently on a series of glycogen standards.

All anthrone tests were performed as follows: 1-ml samples were placed in a number of test tubes in an ice bath and 5 ml anthrone reagent were carefully added. The mixture was vigorously shaken and brought to room temperature. The tubes were placed in boiling water and remained there for exactly 8 minutes after the temperature reached 95 C. The rack of tubes was turned 90° each minute during the boiling process to insure equal heating of the tubes. The tubes were then cooled in tap water and read at 625 millimicrons in a Coleman Jr. spectrophotometer against a blank of 1 ml water and 5 ml anthrone treated otherwise as were the samples. Four serial dilutions of glycogen standard were determined with each lot of samples. The concentrations of the standards were plotted against optical density on regular graph paper and the concentra-

Received for publication January 3, 1961.

* This investigation was supported (in part) by a research grant (PHS–E795) from the Institute of Allergy and Infectious Diseases, of the National Institute of Health, Public Health Service.

tions of the samples were read from this graph.

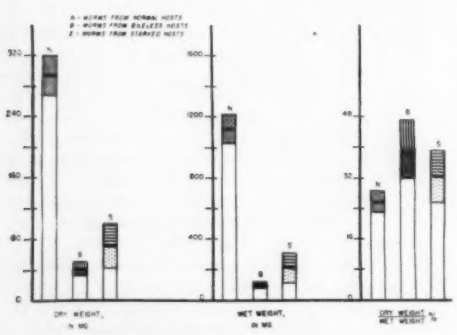
Anthrone reagent was prepared by the method of Fales (1951): 0.4 g anthrone** was dissolved in 200 ml H_2SO_4 (DuPont, A.C.S. Reagent Grade, Sp. Gr. 1.84) in an ice bath. This solution was poured slowly into a mixture of 15 ml 95 percent ethanol and 60 ml distilled water in a flask in an ice bath. The flask was swirled in the ice bath during the addition. Anthrone reagent was kept in a stoppered brown glass bottle in the refrigerator when not in use and fresh reagent was prepared every other day.

Data were analyzed statistically using "Student's" t-test for probability of significance of difference between means; results are given in histograms.

RESULTS

Dry weights, wet weights, and dry weight: wet weight ratios in percent for 27-day-old adult *H. diminuta* from normal, bileless, and starved hosts are given in figure 1. In figure 1 (and in figure 2) mean values are shown by the heavy lines midway in the blocked areas above and below which are indicated two standard errors of the mean.

If "p" values at the 5 percent level (and less) are considered to represent significant differences, then dry and wet weights of worms from bileless and starved hosts are significantly less than the weights of worms from normal hosts. Moreover, worms from bileless hosts are significantly lighter in dry and wet weights than worms from starved hosts. Ratios of dry to wet weight indicate the water content of worms. These ratios show that bileless worms (60 percent H_2O) contain significantly less water ($p<0.01$) than do normal worms (74 percent H_2O), but that starved worms (68 percent H_2O) compared to normal worms ($p>0.05$) and bileless compared to starved worms ($p>0.10$) contain statistically equivalent amounts of water.



EXPLANATION OF FIGURES

FIGURE 1. Comparison of weights of 27-day-old tapeworms from normal, bileless, and starved rats. Mean of each po[illegible]ion indicated by line in center of blocked area. [illegible] S.E.$_M$ are shown above and below the mean va[illegible].

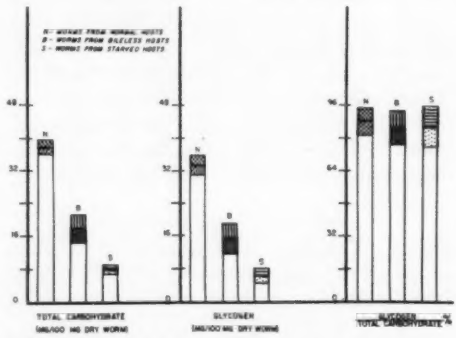


FIGURE 2. Comparison of total carbohydrate, glycogen, and their ratios in lyophilized, 27-day-old tapeworms from normal, bileless, and starved rats Mean values indicated by lines in center of blocked areas. Two S.E.$_M$ are shown above and below the mean value.

Total carbohydrate, glycogen, and the glycogen: total CHO ratios for freeze-dried 27-day-old worms recovered from normal, bileless, and starved hosts are given in figure 2. As is apparent from the figure, worms from bileless and starved hosts contain, respectively, only about one-half or one-fifth as much total CHO and glycogen per 100 mg of dry material as is found in 100 mg of worm from normal hosts. Although depressions in total CHO and glycogen occurred in both bileless and starved worms as compared to normal worms ($p<0.01$), the ratios indicate that percentages of glycogen to total CHO (about 89 percent) remained essentially as they are in normal worms ($p>0.50$).

Since freeze-dried worms from bileless and starved hosts weighed less than 100 mg (on the average) and worms from normal hosts weighed more than 100 mg, it is obvious that absolute amounts of total CHO and glycogen in the three populations varied more than are indicated by carbohydrate contents per equivalent

** Purchased from Law and Company, Consulting and Analytical Chemists, Box 1558, Atlanta 1, Georgia, and prepared by them following a process given in *Organic Syntheses*, Collective Volume I, N. Y. 1941, p. 60.

weights. The magnitude of depletion of total CHO and glycogen in worms from bileless and starved hosts is illustrated when total amounts (mg dry worm × percent carbohydrate) are computed. Normal worms contain 111.8 mg total CHO and 98.5 mg glycogen; bileless worms, on the other hand, contain only 7.7 mg (6.9 percent of amount in normal worms) and 6.7 mg (6.8 percent), and starved worms 5.8 mg (5.2 percent) and 4.6 mg (4.7 percent), respectively, for the same two carbohydrate determinations. Dried bileless worms are only 59.7 percent of the weight of starved ones; yet in absolute amounts they contain 1.3 times as much total CHO and 1.4 times as much glycogen as the starved worms.

DISCUSSION

Results from this study and previous ones (Goodchild, 1958, 1960) indicate that lack of bile exerts profound suppressive effects upon length and weight of *H. diminuta* in rats. Dry and wet weights of tapeworms from bileless hosts fed ad libitum on laboratory chow are significantly less than are those of worms from normal rats; and what is even more striking, worms from bileless hosts, in both dry and wet weights, are lighter than worms from starved hosts after identical periods of treatment. Starvation, thus, seems less detrimental to tapeworms in these regards than does being in an intestinal environment containing exogenous food but lacking bile. Moreover, the water content of worms from bileless hosts (60 percent) is less than the water content of worms from normal hosts (75 percent). The significance of this difference in water content is not yet clear.

Slight differences may be noticed in wet weights of worms used in this study from those given in a previous paper (Goodchild, 1960) for the same populations; dry weights had not yet been determined in the earlier work. The present study used only 9 of 15 worms from normal hosts, 9 of 11 worms from bileless hosts, and 9 of 11 worms from starved hosts. Only worms actually chemically analyzed were used for weight determinations. Some analyses used less than the 9 worms weighed; lack of material prevented complete analysis of the same 9 worms. For example, from bileless hosts 6 worms were analyzed for both total CHO and glycogen, 2 additional worms were analyzed for total CHO only, and 1 additional worm was analyzed for glycogen only.

Ranges of total carbohydrate and glycogen in normal worms found in this study agree rather closely with results obtained by Landt (unpublished) in this laboratory. Landt's analyses performed on lipid-free lyophilized, normal *H. diminuta* yielded 42 percent total CHO and 35 percent glycogen. Results of the present study agree, too, with ranges of percent glycogen in various cestodes compiled by von Brand (1952).

Originally the work had been planned to determine total carbohydrate, glycogen, and glucose in various regions of tapeworms from different types of experimental hosts. Much time was spent in earlier phases of the work in quantitative separation of carbohydrate fractions, but without achieving consistent results. When the method of Fales (1951) was adopted later, however, replication of results on total CHO and glycogen in tissues (freeze-dried rat liver and tapeworms) gave values with less than a 5 percent spread; furthermore, analysis of standards with known amounts of glycogen and glucose yielded 100 percent recovery of total CHO and 95 to 100 percent recovery of precipitated glycogen. As mentioned earlier, four serially diluted standards were run with each set of determinations for carbohydrate. Optical density values for these standards lie on or close to a straight line and values for carbohydrates in the tapeworm tissue were read from graphs made for each run. Table I depicts results from one set of determinations.

TABLE I. *Total carbohydrate (determined by the anthrone method) in five samples each of three normal tapeworms. (Results with four dilutions of a glycogen standard run concurrently are given.)*

Worm No.	Optical density	Mg CHO per ml	Mg worm per ml	Percent CHO
N-17	.440	.06375	.144	44.3
	.412	.05825		40.5
	.417	.05900		41.0
	.420	.05975		41.5
	.441	.06300		43.7
N-19	.401	.05675	.154	36.9
	.417	.05900		38.3
	.409	.05775		37.5
	.412	.05825		37.8
	.401	.05675		36.9
N-20	.282	.03725	.110	33.9
	.295	.03950		35.9
	.290	.03900		35.5
	.288	.03825		34.8
	.292	.03925		35.7
Standard				
.09500 mg/ml	.643			
.07125 mg/ml	.485			
.04750 mg/ml	.342			
.02375 mg/ml	.199			

Analyses of carbohydrate contents of anterior, middle, and posterior thirds of tapeworms were performed in early phases of the study, but are not reported in detail in this paper because the results were not consistent enough; nevertheless, trends seemed to be suggested by the data collected. A consistent pattern of glucose and glycogen distribution in anterior, middle, and posterior thirds of worms from normal hosts appeared evident. Glucose percentage was lowest in the middle third, highest in the posterior third, and intermediate in the anterior third. High concentrations of glycogen and low content of glucose in the middle third seem consistent with the intense reproductive activity in this zone. Read and Rothman (1957) reported a "material staining red with iodine (polysaccharide?) in eggs" of *H. diminuta*. This material would seem to be incorporated in the eggs during the time of their formation in the mature zone which lies roughly in the middle third of the worm. On the other hand, high concentration of glucose and low concentration of glycogen posteriorly may be due to hydrolysis of stored glycogen and loss of glucose from the tapeworm into the intestinal lumen for possible absorption by the host. If so, this loss to the host would be a striking case of biological adaptation to parasitism on the part of this tapeworm, and constitutes a "saving of the host" similar to that reported in certain other endoparasites; for example, von Brand (1952) stated, ". . . the host may be able to utilize at least some of the end products of the parasite's metabolism."

It needs to be stated again, as it was in an earlier paper (Goodchild, 1960), that bileless rats from which tapeworms were recovered gained weight at rates statistically equivalent to unoperated rats. In fact, during the 7 day experimental period normal rats gained an average of 19 ± 5.2 g, bileless rats gained 16 ± 11.0, and starved rats lost 51 ± 3.6. Bileless hosts were thus eating at near normal rates, and tapeworms in these hosts were being constantly exposed to exogenous food. Yet difficulty was experienced in replenishing their stores of carbohydrate; absolute amounts of total CHO and glycogen in worms from bileless and starved hosts gradually declined to nearly the same levels. The carbohydrate uptake mechanism in normal hosts is not too clear; it is less so in bileless hosts. Many studies of in vitro utilization of mono- and disaccharides and in vivo utilization of these sugars and various polysaccharides (cf. brief review in Goodchild, 1960) have given insight into relative efficacy of various carbohydrates as energy sources and as raw materials in glycogenesis. Elucidation of the manner of actual absorption of carbohydrate, whether by phosphorylation or by formation of biliary-carbohydrate complexes, awaits additional studies.

SUMMARY

Total corbohydrates and glycogen have been determined by the anthrone method for freeze-dried groups of the rat tapeworm, *Hymenolepis diminuta*, grown in normal, bileless, and starved Wistar strain rats.

Dried 27-day-old worms maintained for their last 7 days in bileless and starved hosts contained only one-half to one-fifth the amount of total carbohydrate and glycogen per 100 mg as did 27-day-old worms from normal hosts. However, dried worms from bileless and starved hosts weighed on the average less than 100 mg, whereas worms from normal hosts weighed much more. In absolute amounts, tapeworms from normal hosts contained 111.8 mg total carbohydrate, whereas worms from bileless and starved hosts contained only 6.9 percent and 5.2 percent, respectively, of this amount; and worms from normal hosts contained 98.5 mg glycogen, whereas those from bileless and starved hosts contained only 6.8 percent and 4.7 percent, respectively, of this quantity. Worms from bileless hosts were 59.7 percent the weight of starved worms yet contained 1.3 times and 1.4 times as much total carbohydrate and glycogen.

Statistical analyses of the three populations revealed significant differences when comparisons were made of amounts of total carbohydrate and glycogen per 100 mg of dried worm. Differences in the absolute amounts of total carbohydrates and glycogen in the three groups were even more profound.

In 100 mg of dried tissue, worms from normal hosts contained 37.9 ± 0.9 mg total carbohydrate, and worms from bileless and starved hosts would contain 17.8 ± 1.6 mg and 8.1 ± 0.5 mg total carbohydrate, respectively. In the same 100 mg of tissue, worms from normal hosts contained 33.4 ± 1.2 mg glycogen, and worms from bileless and starved hosts would contain

15.5 ± 1.8 mg and 6.4 ± 0.9 mg glycogen, respectively. The glycogen to total carbohydrate ratios in all three groups were not significantly different and ranged between 85.3 percent (for bileless worms) and 88.5 percent (for normal worms).

An evaluation of the accuracy of the anthrone method for carbohydrate determinations in glycogen standards and in tissues is presented.

References

VON BRAND, T. 1952 Chemical Physiology of Endoparasitic Animals. Academic Press, New York, N. Y.

FALES, F. W. 1951 The assimilation and degradation of carbohydrates by yeast cells. J. Biol. Chem. **193**: 113–124.

GOODCHILD, C. G. 1958 Growth and maturation of the cestode *Hymenolepis diminuta* in bileless hosts. J. Parasit. **44**: 352–362.

———— 1960 Effects of starvation and lack of bile upon growth, egg production and egg viability in established rat tapeworms, *Hymenolepis diminuta*. J. Parasit. **46**: 615–623.

HARROW, B., BOREK, E., MAZUR, A., STONE, G. C. H. AND WAGREICH, H. 1960 Laboratory Manual of Biochemistry. W. B. Saunders Co., Philadelphia.

READ, C. P. AND ROTHMAN, A. H. 1957 The role of carbohydrates in the biology of cestodes. IV. Some effects of host dietary carbohydrate on growth and reproduction of *Hymenolepis*. Exp. Parasit. **6**: 294–305.

RESEARCH NOTE

PARASITIC CERATOPOGONIDAE AND SIMULIIDAE (DIPTERA) FROM IMPERIAL COUNTY, CALIFORNIA.

The following collections of parasitic Diptera were made by Raymond E. Ryckman, Robert D. Lee, and Dean Spencer on March 27 to 29, 1955, in the relatively arid northeastern part of Imperial County, California. During daylight hours, jackrabbits (*Lepus californicus*) near Davis Lake were found to be heavily parasitized by *Leptoconops kerteszi* (Kieffer), *Culicoides variipennis* (Coq.), and *Simulium griseum* (Coq.). The extensive parasitism by these flies was evidenced by the many small scars and serum incrusted ears of the rabbits. The collections were made by shooting the rabbits and immediately picking the engorged flies from their ears. Similar collections of *L. kerteszi* and *S. griseum* were also made from the ears *L. californicus* near Midway Well. Distribution of the above mentioned ceratopogonids in California has previously been reviewed (Wirth, 1952, Univ. Calif. Publ. Ent. **9**: 95–266).

While setting mammal traps on the night of March 27, 1955, at the desert oasis of Indian Well, a house finch or linnet, *Carpodacus mexicanus*, flew from her nest in a large *Opuntia Munzii* plant. Small Diptera, their abdomens red with recently engorged blood, were seen running about in the nest and on the eggs. With considerable difficulty some the these specimens were collected and returned to the laboratory for identification; they were subsequently found to be an undescribed species of ceratopogonid, viz, *Culicoides ryckmani* Wirth and Hubert, 1960. During the night, this same species and *Culicoides cockerellii* (Coq.) were taken in a light trap in the same vinicity.

Previous investigations (Ryckman and Ames, 1953, Pan-Pac. Ent. **29**: 163–164; Ryckman, 1960, Ann. Ent. Soc. Am. **53**: 659–661; Wirth and Hubert, 1960, Ann Ent. Soc. Am. **53**: 639–658) indicate that many species of Ceratopogonidae (Heleidae) breed in the larval stages in necrotic pockets in cacti. It now seems quite probable that the cactiphilic *Culicoides* of the Copiosus group feed at night or during crepuscular hours in the desert on roosting or nesting birds. Members of the Copiosus group are quite readily killed by low relative humidity at room summer temperatures in southern California. It is believed that these gnats feed at night when the relative humidity is considerably higher and the temperatures lower than during the day, and when their prospective hosts are quiet.

(This investigation was made possible by Research Grant E-874 from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service.)—RAYMOND E. RYCKMAN, *Department of Microbiology, Loma Linda University, Loma Linda, California.*

RESEARCH NOTE

A QUANTITATIVE FIELD TECHNIQUE FOR DIFFERENTIATING SOUTH AFRICAN *SCHISTOSOMA MATTHEEI* CERCARIAE FROM THOSE OF *S. MANSONI*.

In a previous paper (Rowan, 1957, J. Parasit. 43: 696–697) a method was outlined for the recovery of schistosome cercariae from natural waters. The cercariae, trapped on filter paper, were stained and counted. Stained furcocercous cercariae were easily differentiated from non-furcocercous species, but related furcocercous cercariae could not be distinguished. The following buffer staining technique extends the versatility of the original method to permit differentiation of two of the previously indistinguishable species.

1. Cercariae are collected on S and S 404 filter paper by pressure filtration or suction (see Rowan and Gram, 1959, Am. J. Trop Med. Hyg. 8: 630–634), whichever method best suits local conditions.
2. The filter paper is transferred face up to a shallow pan containing just enough brom thymol blue solution to saturate the paper. Excess of solution is detrimental. To prepare the stain dissolve 0.1 g of brom thymol blue in 10 ml of 95 percent ethyl alcohol and dilute by adding 90 ml of 0.1 M phosphate buffer (Na_2HPO_4–KH_2PO_4) at pH 8.0. The solution can be used immediately and keeps well for several days.
3. The paper is steamed in the field by holding the pan over an improvised alcohol stove. Heating is continued until most of the water has evaporated but the paper is not yet dry.
4. The moist paper is stored in a suitable carrying container for transport to the laboratory. The paper dries gradually en route and when completely dry the cercariae appear blue-black on an orange-yellow background.
5. A total count of the cercariae is made from the dry paper at any time within 72 hours of the time of preparation. The furcocercous cercariae are circled with a pencil. When only a few cercariae appear on the paper these are cut out and transferred to a petri dish for further processing.
6. The paper is immersed in distilled water for 1 minute.
7. Distilled water, 5 seconds.
8. Distilled water, 5 seconds.
9. 0.01 M phosphoric acid, 15 seconds.
10. Distilled water, 5 seconds.
11. 0.01 M phosphate buffer, pH 7.0, 5 seconds.
12. 0.01 M phosphate buffer, pH 7.0, 45 seconds.
13. Distilled water. Observe. The cercariae of laboratory reared and wild *S. mansoni* are a uniform orange against a white background if the technique is properly carried out. A pale and diffuse greenish area may appear posterior to the acetabulum when papers are processed immediately after preparation or when the final buffer solution is above pH 7.0. The greenish area is rarely seen in *S. mansoni* when papers are allowed to stand for from 24 to 72 hours before processing. Laboratory reared *S. mattheei* cercariae are also orange but possess a solid knot of green posterior to the acetabulum. The green coloration appears rapidly in *S. mattheei* on addition of pH 7.0 buffer solution, is more intense than any green which might appear in *S. mansoni* cercariae treated similarly, and persists longer when the papers are transferred to distilled water (step 13). The difference is apparently species specific and is not influenced by the age or sex of the cercariae.

The technique does not provide completely reliable differentiation of individual cercariae. Damaged cercariae stain erratically. However, the technique can be used to typify a wild population of cercariae (e.g. 25 percent *S. mattheei*, 75 percent *S. mansoni*). The method has been field tested and is considered practical where the importance of the information warrants its use.

The identity of the green staining tissue in *S. mattheei* could not be determined. Presumably, the dye, which becomes green and then blue the higher the pH, is responding to localized conditions in the cercarial body, possibly reacting with proteins which, at that location in *S. mattheei* may have a markedly different isoelectric point from those in a similar position in *S. mansoni*. If this is so, variations in this type of buffer staining, by revealing protein differences between cercariae, could provide a means of separating other structurally similar species.

This investigation was done at the Bilharzia Research Station of the Council for Scientific and Industrial Research (C.S.I.R.), Nelspruit, Union of South Africa, and was sponsored jointly by the C.S.I.R. and the U. S. Public Health Service, National Institute of Allergy and Infectious Diseases, Research Grant E-3213 S_1.—WILLIAM B. ROWAN, *Department of Biology, Middlebury College, Middlebury, Vermont.*

SOME PHARMACOLOGICAL ASPECTS OF A TOXIC SUBSTANCE FROM OOCYSTS OF THE COCCIDIUM *EIMERIA TENELLA*[*]

M. T. Rikimaru, F. T. Galysh, and R. F. Shumard[**]
The School of Pharmacy and The Agricultural Experiment Station,
North Dakota Agricultural College, Fargo

Becker (1934) cited the possibility that a toxic substance may be formed and absorbed during the height of infection with coccidia. Chapman (1929) was unable to demonstrate the production of a toxin by rabbit coccidia and believed that the etiology may be due to the production and absorption of a toxic substance or substances produced by bacteria at the site of injury.

Shumard and Eveleth (1955) reported that a substance removed from the intestinal tract of a lamb moribund with experimentally induced coccidiosis and injected intravenously into a rabbit and a young chicken produced symptoms of labored breathing, depression, anorexia, and ataxia. The rabbit succumbed within 12 hours; the chicken recovered and appeared normal after two days. Shumard (1957) proposed the view that a toxic substance from the intestines of lambs moribund with coccidiosis produced physiological reactions in rabbits similar to an endotoxin. A Shwartzman-like phenomenon of local tissue reactivity was observed with sublethal doses. Extensive localized lesions of the visceral organs were observed. Single lethal doses produced circulatory changes (evidenced by alternate constriction and dilation of ear blood vessels), anorexia, ataxia, hyperthermia, and hyperventilation. Death occured in 8 to 24 hours. This evidence indicates the possibility that some of the pathogenesis of coccidiosis may be due to toxic substance or substances produced by the coccidian. In accordance with this hypothesis, Bertke (1955) had suggested that histopathological changes observed in organs not primarily affected by the coccidian, *Eimeria tenella*, might be due to a toxic substance that was produced by or released through the action of the parasite. Levine and Herrick (1954) and Schildt and Herrick (1955) described early physiological changes in responses of skeletal and smooth muscle which indicate involvement of other than simple mechanical damage due to the parasite.

Burns (1959) reported on the lethal effect of *Eimeria tenella* extract in rabbits. Extracts from the coccidial oocysts and the cecae of chickens infected with this parasite were usually lethal when injected intravenously or intraperitoneally. The observed toxin, when injected in sub-lethal doses into rabbits, induced the formation of antibodies that neutralized the toxin. The injection of these toxic extracts into chickens did not produce toxin-neutralizing antibodies, nor were the chickens protected from subsequent coccidial infection.

MATERIALS AND METHODS

The oocysts used for infection were from the same strain of *Eimeria tenella* cited by Burns (1959). Sporulated oocysts were used to infect 6- to 8-week-old coccidia-free chickens. Oocysts collected from the cecal mucosa were sporulated in 2.54 percent potassium dichromate. Ninety-five percent sporulation of the oocysts was accomplished by using aeration and sporulating for 72 hours. The oocysts were then washed free of the potassium dichromate solution with the aid of centrifugation and subjected to peptic digestion using 5.0 g of commercial pepsin and 7 ml of concentrated hydrochloric acid brought up to total volume of 1 liter with distilled water. The mixture, constantly agitated by a magnetic stirrer, was allowed to digest for 24 hours at 37 C. The oocysts were then washed free of the digestive solution with the aid of centrifugation. A sterile saturated sodium chloride solution was added to the residue to aid in the separation of the oocysts from the extraneous matter. Separation was effected more fully by centrifugation. The oocysts were then washed free of the sodium chloride solution, transferred to a tissue homogenizer tube, and again centrifuged at 1000 r.p.m. The tube was then immersed in an ice-water bath and the oocysts macerated with a loose fitting pestle until microscopic examination revealed that at least 90 per-

Received for publication August 18, 1960.

[*] Published with the approval of the director of the North Dakota Agricultural Experiment Station, Fargo.

[**] Present address: Sli Lilly and Company, Agricultural Research Center, Greenfield, Indiana.

cent of the walls of the oocysts present were ruptured. The majority of the sporozoites did not seem to be affected by this treatment, but remained essentially intact. Sterile physiological saline solution was added until a final concentration of 583,000 ground oocysts per ml was obtained. This mixture was then filtered 5 times through sterile, medium-porosity, sintered-glass filters and 3 times through sterile, fine-porosity, sintered-glass filters, and then transferred aseptically to a rubber-stoppered bottle. The resultant solution was clear. The filtrate was tested for contaminants by culturing on agar slants aerobically and in agar culture plates anaerobically. No growth was observed after 3 days incubation at 37 C. The toxic dose level of the material was established using two rabbits. Preliminary trials utilizing the same number of oocysts indicated that 5 ml of this substance (equivalent to a concentration of approximately 2,900,000 ground oocysts) injected intravenously was consistently fatal to rabbits.

Blood pressures of six non-anesthetized rabbits were determined. Under anesthesia, the carotid artery was isolated and ligated at the distal end. A polyethylene cannula, previously prepared with a heparin solution, was inserted into the artery, tied securely into position and clamped off. The incision was then closed with small metal clips allowing the cannula to protrude outside of the body. The rabbits were allowed to recover from anesthesia and surgical trauma for 1.5 to 2 hours. After recovery, the cannula was attached to a polyethylene tube (filled with 8 percent sodium citrate) connected to a mercury manometer. After 15 to 20 minutes of normal tracings, 5 ml of the toxic substance was injected into the marginal ear vein. The animals were conscious and in a normal upright position in a well ventilated rectangular box with only their heads protruding during the entire recording procedure. The rabbits appeared normal and comfortable previous to injection.

For electrocardiographic studies, a sling fabricated from cloth, with holes for legs and head, was used to suspend a rabbit between two ring-stands. The medial-proximal aspect of all four legs was shaved and the electrodes from a Beck-Lee electrocardiograph attached. The animals appeared to have no discomfort in this position. Normal patterns were obtained and then 5 ml of the toxic substance was slowly injected into the marginal ear vein. Recordings were taken at 30-minute intervals and continuously during convulsions prior to death.

Blood glucose was determined by the Somogyi (1945) method. Blood was drawn from the ear vein using a syringe moistened with 1 drop of heparin solution. Samples were obtained prior to injection of the toxic substance at 30 minutes and then at 60-minute intervals. The last sample was taken by cardiac puncture immediately after respiration ceased. Temperatures were taken rectally by means of a modified thermocouple prior to the withdrawal of the blood during blood glucose determinations.

RESULTS

General

Following injection of the toxic material into the marginal ear vein of the rabbit, these observations were made: Immediate dilation of the blood vessels of both ears, followed by alternate constriction and dilation of the vessels until death. The onset of respiratory distress began with hyperventilation proceeding gradually to that of labored breathing and finally respiratory arrest. Prior to respiratory arrest the animal emitted cries and exhibited tetanic-type convulsions. Rigor mortis was complete within a few minutes.

Gross post-mortem examinations revealed yellow exudate present in the lumen of the intestine. The contents of the intestine were extremely gaseous. The musculature of the gastrointestinal tract appeared to be in a state of atonic contraction. The pyloric sphincter was closed. A generalized serous exudate occurred throughtout the viscera.

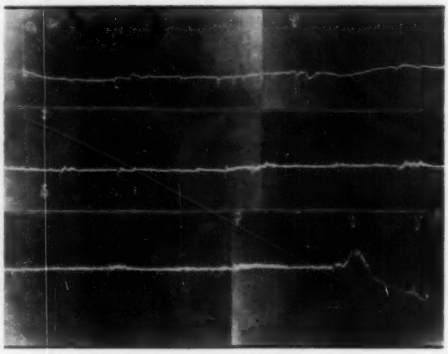


FIGURE 1. Normal blood pressure tracing from 1 to 3. At 2 the blood pressure was 105 mm Hg. At 3, 5 ml of the toxic oocyst substance was injected into the marginal ear vein. A slight drop in pressure occurred, followed by a gradual rise. At 4 the pressure was 110 mm Hg. Abnormalities probably produced by vagal effects appeared and corresponded to the vasoconstriction and dilation observed in the ear vessels. These vagal effects gradually increased until, at 5, they were rather intense. At 5 the blood pressure was 110 to 115 mm Hg, the fluctuation being rather constant. There was a marked rise in blood pressure 6, 130 minutes after the toxic substance was injected, evidently produced by increased intensity of ventricular contraction. The pressure dropped to 40 mm Hg 138 minutes after injection (7). Respiration ceased at 140 minutes (8).

Effect on Blood Pressure

When the toxic substance was injected into the marginal ear vein, an immediate drop in blood pressure occurred (fig. 1). Following this there was a gradual increase in pressure to slightly above normal. Fluctuations and irregularities began soon after recovery from the initial drop, but were much more apparent as terminal toxicity approached. Respiration was observed to cease before the final fall in blood pressure, indicating that respiratory failure preceded cardiac arrest.

Effects as Demonstrated by Electrocardiography

Electrocardiograph recordings are depicted in plate I. (Leads I, II, and III were recorded; those of lead I are reported here.) The first irregularity noted in the tracings was a voltage decrease in the T wave and QRS complex. The ventricular rate increased and then gradually decreased in intensity. Voltage of the T wave continued to decrease; eventually inversion of the T wave and extreme distortion of the QRS complex took place. At approximately 2 hours following injection of the toxic substance compensatory reversion occurred resulting in a normal ECG pattern with depressed voltage. As convulsions occurred, voltage irregularities were produced, presumably independent of cardiac output. Complete auricular blockage occurred and the strength of ventricular contractions was increased upon respiratory arrest. Disturbances in ventricular conduction and refractory periods were noticeable, the ventricular rate indicating complete auricular-ventricular block and assumption of slow pacemaker activity. This was immediately followed by increased ventricular rates for a short period of time, probably due to extrasystolic action. Complete cardiac arrest took place within 3 minutes following respiratory failure.

TABLE I. *Body temperatures (F) of rabbits following intravenous injection with* Eimeria tenella *oocyst extract.*

Time (min.)	Control		Toxic substance		
0	103.0	101.8	102.0	101.2	102.0
30	103.5	101.3	101.0	102.6	102.5
90	103.4	102.4	103.6	104.5	103.2
150	103.5	102.0	103.2	103.2	103.4
210	102.8	103.3	104.5	105.8	105.3
270	102.6	101.0	104.2	106.4	104.0

Effects on Body Temperatures

Body temperatures of the rabbits intravenously injected with the oocyst extract were elevated beyond the normal range. Hyperthermia was first recorded at 90 minutes following injection and persisted until death occurred (table I).

Effects on Blood Sugar

Initial blood glucose levels were normal (table II). Hyperglycemia developed between 30 and 90 minutes post injection and persisted for some time. Approximately 2 hours before death occurred, a hypoglycemic condition developed and at death blood glucose was nearly depleted.

TABLE II. *Blood glucose (mg glucose/100 ml) of rabbits following intravenous injection with* Eimeria tenella *oocyst extract.*

Time (min.)	Control		Toxic substance		
0	65	52	75	65	65
30	69	55	85	65	69
90	69	55	138	100	96
150	71	58	143	130	109
210	71	65	71	120	130
270	69	60	—	82	138
330	—	—	23*	—	—
370	—	—	—	5*	—
530	—	—	—	—	8*

* Death occurred at this time.

DISCUSSION

The physiological disturbances produced during the conduction of these studies resemble those reported in studies of endotoxins (Thomas, 1954). Shumard (1957) reported that the pathology produced in a rabbit, by intravenous injection of a substance extracted from the intestine of a lamb moribund with coccidiosis, resembled that produced by bacterial endotoxins. The substance described in the present paper and endotoxins, in general, produce the following: Hyperglycemia followed by hypoglycemia, febrile response; circulatory abnormalities, including vasoconstriction and dilation of rabbit ear blood vessels; electrocardiographic abnormalities in rabbits, and effects on biochemical activities.

The alternate constriction and dilation of blood vessels may have produced some cardiac ischemia giving rise to an increase in the refractory period or depression of conduction velocity of the cardiac muscle. Irregularities of the T wave are primary indicators of abnormalities of the cardiac musculature. Depression or overstimulation of cellular metabolism or infectious processes can produce such changes in the T wave. Toxicities tend to affect the T wave before they affect other portions of the electro-

cardiogram; however, if the toxicities become severe enough they can cause other injuries and eventually can distort the QRS complex (Guyton, 1956). The extreme toxicity of the material investigated was revealed by drastic distortions of the QRS complex. The absence of the P wave, or depolarization of the atria, indicates cessation of a functional sino-auricular node (S-A block) which apparently occurred following respiratory arrest and, therefore, may have been only an indirect result of the oocyst extract.

Increases in blood sugar in chickens infected with *E. tenella* have been described by Herrick (1933, 1939), Pratt (1940), and Waxler (1941). Pratt (1941) attributed the hyperglycemia to formation of glucose from muscle lactic acid. Daugherty and Herrick (1952) presented the hypothesis that accumulation of blood glucose during *E. tenella* infection was due in part to interference with normal phosphorylation. Hyperglycemia (the cause of which is unknown) was noted in all injected rabbits in this study.

Whether the toxic substance described is part of the etiology of cecal coccidiosis in the domestic fowl remains conjectural.

SUMMARY

Toxic substances from the oocysts of the coccidium, *Eimeria tenella,* produced death when injected intravenously into rabbits. The following effects were observed in pharmacological investigations with this substance in the rabbit: an immediate drop in blood pressure with a subsequent increase followed by a marked increase in pressure just prior to death; abnormalities in electrocardiographic tracings, principally inversion of the T wave, distortion of the QRS complex, and absence of the P wave at respiratory arrest; pyrogenic responses resulting in elevated body temperature; hyperglycemia followed by hypoglycemia and blood-sugar depletion at death.

LITERATURE CITED

BECKER, E. R. 1934 Coccidia and coccidiosis of domesticated, game and laboratory animals and of man. Collegiate Press, Ames, Iowa.

BEETKE, E. M. 1955 Pathological effects of coccidiosis caused by the protozoan parasite *Eimeria tenella* in chickens. Doctoral Thesis. University of Wisconsin Library.

BURNS, W. C. 1959 The lethal effect of *Eimeria tenella* extracts on rabbits. J. Parasit. **45**: 38–46.

CHAPMAN, J. 1929 A study of coccidiosis in an isolated rabbit colony. Am. J. Hyg. **9**: 389–429.

DAUGHERTY, J. W. AND HERRICK, C. A. 1952 Cecal coccidiosis and carbohydrate metabolism in chickens. J. Parasit. **38**: 298–304.

GUYTON, A. C. 1956 Textbook of Medical Physiology. W. B. Saunders Company, Philadelphia.

HERRICK, C. A. 1933 Some physiological effects of coccidia on chickens. J. Parasit. **20**: 123–124.

HERRICK, C. A. 1939 Changes occurring in blood of chickens during cecal coccidiosis. J. Parasit **25** (supp.): 34–35.

LEVINE, L. AND HERRICK, C. A. 1954 The effects of the protozoan parasite *Eimeria tenella* on the ability of the chicken to do muscular work when its muscles are stimulated directly and indirectly. J. Parasit. **40**: 525–531.

PRATT, I. 1940 The effect of *Eimeria tenella* (Coccidia) upon the glycogen stores of the chicken. Am. J. Hyg. **34**: 31–37.

PRATT, I. 1941 The effect of *Eimeria tenella* (Coccidia) upon the glycogen stores of the chicken. Am. J. Hyg. **34**: 54–61.

SCHILDT, C. S. AND HERRICK, C. A. 1955 The effect of cecal coccidiosis on the motility of the digestive tract of the domestic fowl. J. Parasit. **41** (supp.): 18–19.

SHUMARD, R. F. 1957 Ovine coccidiosis: incidence, endotoxin (?) and treatment. J. of Am. Vet. Med. As. **131**: 559–560.

SHUMARD, R. F. AND EVELETH, D. F. 1955 A preliminary report on a toxic substance produced by *Eimeria* spp. in a lamb. Rept. of Conf. on Parasites and Parasitic Dis. of Dom. Ruminants. Agr. Res. Ser. U.S.D.A. 6–7.

SOMOGYI, M. 1945 Determination of blood sugar, J. Biol. Chem. **160**: 69–73.

THOMAS, L. 1954 The physiological disturbances produced by endotoxins. Ann. Rev. Physiol. **16**: 467–490.

WAXLER, S. H. 1941 Changes occurring in the blood and tissues of chickens during coccidiosis and artificial hemorrhage. Am. J. Physiol. **134**: 19–26.

Explanation of Plate I

Figure numbers are in the lower left, time of reading in upper left, and ventricular rates in lower center of each figure.

Figure 1. Normal ECG tracing prior to injection of toxic oocyst material. Ventricular rate = 280 per min. Normal P wave, QRS complex, and T wave.

Figure 2. 30 minutes. Note voltage decreases in T wave and QRS complex. Ventricular rate = 360 per min.

Figure 3. 60 minutes. Note lack of voltage in T wave indicating disturbances in repolarization of myocardium and initiation of sinus arrhythmias. Ventricular rate = 300 per min.

Figure 4. 90 minutes. Note initiation of inverted T waves and extreme distortion of QRS complex. Effects may indicate disturbances in conduction or increase in refractory period of ventricular muscle. Ventricular rate = 240 per min.

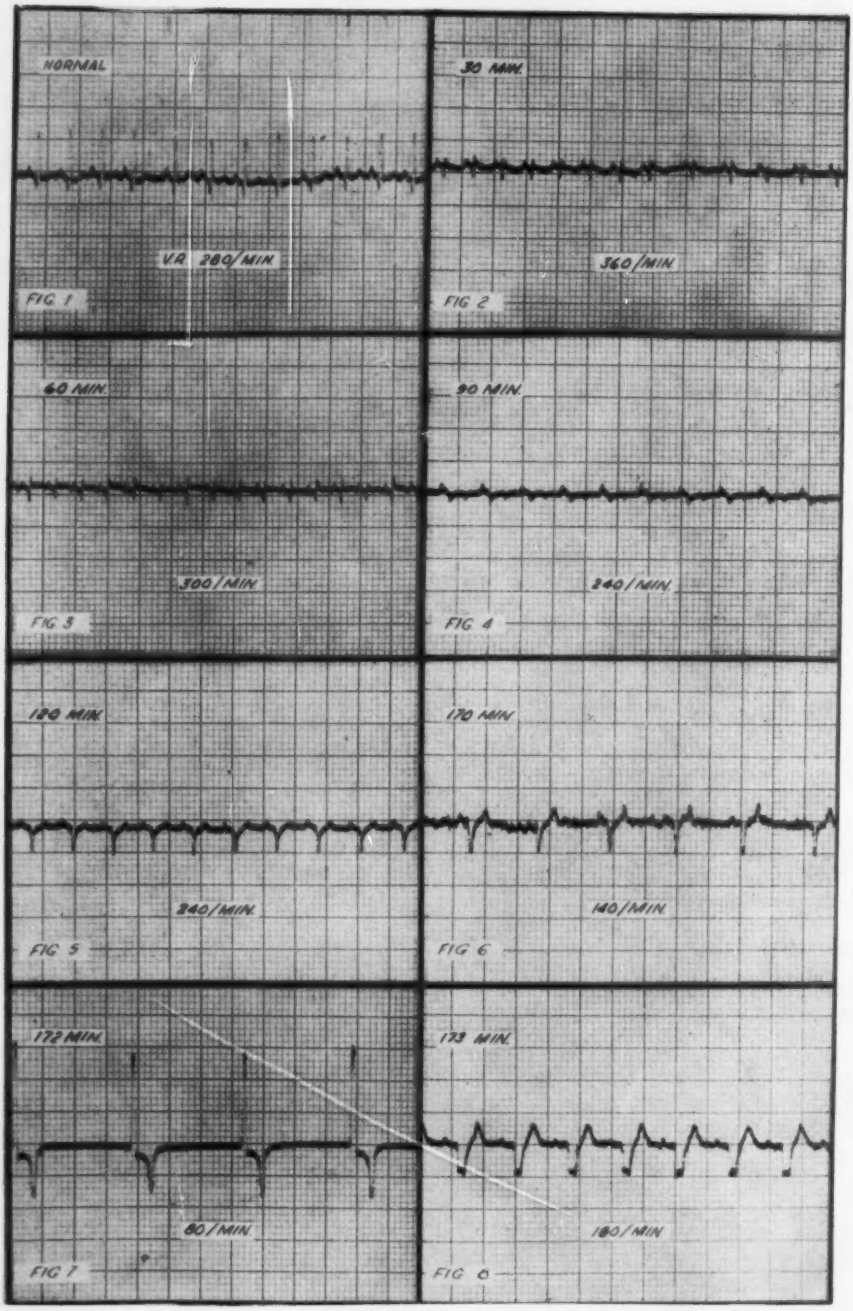
Figure 5. 120 minutes. ECG has reverted to essentially a normal pattern with some depression of voltage characteristics. Ventricular rate = 240 per min.

Figure 6. 170 minutes. Voltage fluctuations are accounted for by currents produced by simultaneously occurring convulsive activity of the animal. Ventricular rate = 140 per min.

Figure 7. 172 minutes after injection of the toxic substance, immediately following respiratory arrest. Note complete S-A block (absence of P wave), increased strength of ventricular contraction (extremely high voltages in the QRS complex) and disturbances in ventricular conduction and refractory period (inverted T wave). The ventricular rate of 80 per minute indicates complete A-V block and assumption of slow pacemaker activity by the ventricles. The increased force of ventricular contraction produced an increased cardiac output as evidenced by blood pressure rise just before death (see Fig. I).

Figure 8. 173 minutes. Respiration has ceased and extrasystoles (biphasic QRS complex) are evident. Ventricular rate = 180 per min. Following this the ventricular rate diminished to 100 per min at 174 minutes. At 175 minutes ventricular fibrillation occurred followed by complete cardiac arrest.

PLATE I


NORMAL
V.R. 280/MIN
FIG. 1
30 MIN.
360/MIN.
FIG. 2
60 MIN.
300/MIN.
FIG. 3
90 MIN.
240/MIN.
FIG. 4
120 MIN.
240/MIN.
FIG. 5
170 MIN.
140/MIN.
FIG. 6
172 MIN.
80/MIN.
FIG. 7
173 MIN.
180/MIN.
FIG. 8

STUDIES OF THE AGE AND SEASONAL VARIATIONS IN THE BLOOD AND BONE MARROW PARASITES OF A SERIES OF BLACK-BILLED MAGPIES

ROBERT M. STABLER
Colorado College, Colorado Springs

Reports of the blood parasites of birds are extensive. According to Manwell (1955a), however, there have been very few attempts to study the relative incidence of malaria and other protozoan infections of the blood in juveniles, or to discover seasonal variations in the incidence of such infections. Herman (1938) reported on the incidence of *Haemoproteus* in some juvenile and adult chipping sparrows. Manwell (1955b) compared the blood parasites of juvenile and adult robins from New York State and the high Rockies. Coatney and Jellison (1940), Wagner (1946), and Hagen and Olsen (1957), all reported on the hematozoa of black-billed magpies of various age levels. None of these authors, however, too clearly defined the age implications of such terms as "nestling," "fledgling," "juvenile," "immature," and "adult." In a very extensive study of the plasmodia of Kern Co., Calif., birds, Herman et al (1954) noted the organisms in three well-defined age groups of 3 species of birds. Similar observations on the parasites of Canada geese in specific age categories were made by Levine and Hanson (1953), Wehr and Herman (1954), and Hanson et al (1956).

Even less has been published on seasonal variations in parasite incidence. Jordan (1943) noted some seasonal influence on *Haemoproteus* and malaria infections in 3 species of birds. Manwell (1955a) noted seasonal incidence of blood parasites in the song sparrow. Considerable year-to-year incidence data were reported by Herman et al (1954).

The writer has accumulated data relative to the age and seasonal variations in the blood and bone marrow parasites of certain black-billed magpies.

MATERIALS AND METHODS

The birds were black-billed magpies (*Pica pica hudsonia*), collected within a 10-mile radius of Colorado Springs, Colorado. They represent four age groups. Group I: 29 birds 7 to 12 days old, from 7 nests. These ranged from fledglings with huge abdomens, legs as yet unable to support them, feathers quite evident along the tracts, to birds now pretty well feathered, abdomens much smaller, and with legs capable of supporting the body. Group II: 30 birds 21 to 30 days old, also from 7 nests. These were youngsters on either side of nest-leaving time, which is some 25 days after hatching (Linsdale, 1937). Even in the nest they were well feathered, quite capable of running when removed, and with tail feathers some 2 inches long. Those in the upper age range here were outside the nests, able to make short flights from limb to limb, and quite agile at scrambling among the branches. Their tails were about 5 inches long. Group III: 31 birds 42 to 56 days old. These magpies flew well and at the 8-week stage appeared virtually as adept as their parents. They were full size, lacking only a bit more growth of the tail feathers. They followed the adults over the countryside, calling loudly for their food, the bulk of which they still seemed to prefer obtaining from their parents. Group IV: These were the 87 birds classed as adults, all being 8 months or more in age.

All group I birds were secured from the nests. They were taken between 24 May and 10 June 1959 and 1960. Group II birds were about equally divided between those still in the nests and those which had left them. Of the latter, about half were caught by hand, the remainder being shot. These birds were taken between 30 May and 14 June 1959 and 1960. All group III and IV birds were secured by shooting. The former were taken between 18 June and 9 August 1959 and 1960, the latter mainly from February to September, the same years.

Once in hand, the birds were examined as follows. All live birds were blood filmed from a snipped toe nail. This involved all birds of group I, some three-quarters of group II birds, and a few birds in groups III and IV which had only been winged. The blood of all dead birds was filmed from the heart or from one of the large thoracic vessels. The films were stained in Giemsa's stain and subsequently carefully searched for hematozoa.

Eighty-seven adult birds were examined in a study of the seasonal influence on parasitism. Of

Received for publication August 24, 1960.

these, 55 taken during late winter, spring, and summer months were also given a fresh bone marrow examination for trypanosomes, so that they might be used in comparison with the subadults. Thirty-two adults were given a blood examination only. In marrow examination, a femur was removed, its proximal tip cut off, and the entire marrow extracted. This was immediately placed in Ringer's solution and searched for the flagellates. All birds in groups I, II, and III were given marrow examinations in addition to their blood films. The live birds were blood filmed, killed by thoracic compression, and examined at once for marrow parasites. The shot birds were studied between 1 and 5 hours after death.

ACKNOWLEDGMENTS

Many thanks are due the following: Dr. K. C. Emerson, Arlington, Virginia, for identifying the magpie lice; Dr. G. M. Kohls, Rocky Mountain Laboratory, Hamilton, Montana, for checking the associated ticks; and Dr. Alan Stone, U. S. National Museum, Washington, D. C., for identifying the magpie hippoboscids.

OBSERVATIONS

Table I shows the incidences and percentages for the organisms found in the various age groups of the 145 magpies which had both blood and bone marrow examinations. It need only be emphasized that very few magpies under 12 days of age showed any blood or bone marrow parasites, that the group III subadults showed by far the most *Haemoproteus* (nearly 100 percent), that *Leucocytozoon* was relatively common in all age groups, and that microfilariae were found only in birds 6 months or more in age. Also, whereas blood trypanosome incidences were uniformly low, marrow trypanosome percentages were relatively quite high.

The relationship of season to parasite incidence for 87 adult magpies is shown in table II. Unfortunately, the fall season is represented by only 9 birds. Though admittedly suffering from smallness of numbers, the data from these are suggestive. It is noteworthy that the highest *Haemoproteus* incidences were in the summer, the *Leucocytozoon* and microfilariae were relatively common at all seasons, and that no bird was found harboring trypanosomes after June. Furthermore, the only two birds showing *Plasmodium* were collected in the summer months.

Certain adult magpies were shot which were associated with known sets of offspring. The parasites of 8 such parents and their respective young are shown in table III. In general, there does not appear to be too high a correlation between the parasite population of these young birds and that of the available parents.

Various ectoparasites were removed from the group III and IV birds. These included lice hippoboscids (*Ornithoica vicina*). None of these (*Myrsidea picae*), ticks (*Argas persicus*), and was noted on the birds of the 2 younger categories. All nest areas were within relatively short distances of running streams. An examination of the undersides of stones from these streams revealed numerous larval forms of the buffalo gnat (Simuliidae).

DISCUSSION

From the foregoing it appears that infection with certain blood inhabiting protozoa takes place early in the life of the newly hatched black-billed magpie. The absence of microfilariae in the subadults does not necessarily mean that these birds are free of the filarial worms. It may simply mean that the prepatent period is longer than the 56 days of subadult examination.

The results reported here are difficult to compare with those of other workers. In this study the birds comparable with the "nestlings" and "fledglings" of other authors are separated into 2 age groups (I and II). Some of the older birds of group II, which were out of the nests, would probably have been considered as "immatures" or "juveniles" in other reports. Wagner (1946) in Washington, and Hagen and Olsen (1957) in Colorado, reported their "fledgling"

TABLE I. *Parasite numbers and percentages in the blood and bone marrow of 145 black-billed magpies arranged in 4 age groups.*

Group	Age of group	No. per group	Neg. (B)		H		L		M		T(B)		T(M)	
			No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.
I	7–12 da.	29	25	86.2	1	3.4	4	13.8	0	0.0	0	0.0	2	6.9
II	21–30 da.	30	6	20.0	17	56.7	14	46.7	0	0.0	3	10.0	21	70.0
III	42–56 da.	31	0	0.0	30	96.8	21	67.7	0	0.0	4	12.9	20	64.5
IV	6 mos. or more	55	3	5.5	10	18.2	44	80.0	43	78.2	5	9.1	47	85.5

Neg. (B), negative blood film; H, *Haemoproteus*; L, *Leucocytozoon*; M, microfilaria; T(B), *Trypanosoma* on blood films; T(M), *Trypanosoma* in bone marrow.

TABLE II. *Parasite numbers and percentages in the blood only of 87 adult black-billed magpies arranged according to season.*

Months	No. per season	Neg.		H		L		T		P		M	
		No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.
Jan. Feb. Mar.	42	3	7.1	6	14.6	31	73.8	1	2.4	0	0.0	33	78.5
Apr. May Jun.	18	0	0.0	8	44.4	15	83.3	5	27.7	0	0.0	14	77.7
July Aug. Sept.	18	1	5.5	17	94.4	9	50.0	0	0.0	2	11.1	2	11.1
Oct. Nov. Dec.	9	4	44.4	0	0.0	2	22.2	0	0.0	0	0.0	3	33.3

Neg., negative blood; H, *Haemoproteus*; L, *Leucocytozoon*; T, *Trypanosoma*; P, *Plasmodium*; M, microfilaria.

and "nestling" magpies as having high *Haemoproteus* percentages, 73.3 and 72.2, respectively. These figures are substantially higher than the present data from similar age groups.

Wagner noted no *Leucocytozoon* in five "immature" magpies, while Hagen and Olsen reported their highest percentage at this age level. The present study shows all age groups infected, with the highest incidence in the adults. Wagner reported *Trypanosoma* only in his adults. The current data agree with Hagen and Olsen in showing a low incidence of blood trypanosomiasis at most age levels. Hagen and Olsen also noted microfilariae in some "immatures" (12.5 percent). The present results agree with Wagner in indicating them only in the adult magpies.

In other bird species, Manwell (1955b) reported microfilariae from subadult robins in New York and Colorado. In Cape Cod chipping sparrows, Herman (1938) found the adults 2.5 times as infected with *Haemoproteus* as the juveniles.

In the work on geese, Levine and Hanson (1953) and Wehr and Herman (1954) indicated that *Leucocytozoon* was acquired early in the gosling life of birds from Illinois, Michigan, and Utah, and showed a very low incidence in yearlings and adults. *Haemoproteus* appeared at a low incidence in all age levels. Hanson et al (1956) reported *Sarconema eurycerca* as absent in juveniles, but present in older Illinois geese, whereas *Ornithofilaria* sp. showed the highest incidence in the younger birds.

In reviewing published reports it becomes obvious that the number and type of bird, the location of the survey, the time of year of the study, and even the investigator, exert considerable influence on the outcome. The variations in these several factors make direct comparison of such results hazardous.

While the present findings on parasite incidence of siblings associated with specific parents are few, they seem to be the first of their kind and as such serve as a starting point.

One always wonders whether or not associated ectoparasites are responsible for the transmission of the existing hematozoa. Found here were lice, ticks, and hippoboscids, with a ready source of simuliids nearby. Bennett and Fallis

TABLE III. *Relationship of parasite incidence between particular parents and their respective offspring.*

	Parents							Offspring						
Group	No. per nest	No. neg. (B)	H	L	M	T(B)	T(M)	No. per nest	No. neg. (B)	H	L	M	T(B)	T(M)
I	1	0	+	+	+	0	+	4	4	0	0	0	0	0
	1	0	+	+	+	+	+	5	1	2	2	0	0	5
	2	0	0	+	0	0	+							
			0	+	0	0	0	4	1	3	2	0	0	4
II	1	0	0	0	+	0	0	4	0	4	0	0	1	4
	1	0	0	+	+	+	+	4	0	4	2	0	2	4
	2	0	0	+	+	0	+							
			0	+	0	0	+	6	2	1	4	0	0	0

0, parent negative; +, parent positive; No. neg. (B), number with negative blood; H, *Haemoproteus*; L, *Leucocytozoon*; M, *Microfilaria*; T(B), *Trypanosoma* on blood films; T(M), *Trypanosoma* in bone marrow.

(1959) suggested that infection in juvenile birds began early in June and continued for several weeks. They concluded from an ecological analysis in Algonquin Park, Canada, that *Haemoproteus* was probably transmitted by heleids (Ceratopogonidae) rather than by hippoboscids and that simuliids were important vectors for trypanosomes as well as for leucocytozoa.

It appears that the tick (*Argas persicus*) constitutes a new record for the State of Colorado (Dr. C. M. Kohls: personal communication).

SUMMARY

1. A study has been made of incidences of the blood and bone marrow parasites of the black-billed magpie (*Pica pica hudsonia*) as variously related to the age of the bird and to the season during which it was examined. All birds were collected within a 10-mile radius of Colorado Springs, Colorado.

2. One hundred forty-five magpies from four age groups were given blood and bone marrow examinations. The age categories were: Group I, 29 birds 7 to 12 days old; group II, 30 birds 21 to 30 days old (magpies leave the nest when about 25 days old); group III, 31 birds 42 to 56 days old; group IV, adult birds 6 months or more in age.

3. Eighty-seven adult birds were studied to determine the relationship of season to blood parasite incidence. Forty-two winter birds (Jan., Feb., Mar.), 18 spring birds (Apr., May, June), 18 summer birds (July, Aug., Sept.), and 9 fall birds (Oct., Nov., Dec.) were examined.

4. The incidences and percentages for *Haemoproteus, Leucocytozoon, Plasmodium,* microfilariae, and *Trypanosoma* were variously noted for the birds of the 2 studies. The trypanosomes were looked for in both blood and bone marrow.

5. A few adult magpies were collected which were associated with specific sets of nestlings (groups I and II). The relationships of the parasites of parents and offspring were noted.

6. Lice (*Myrsidea picae*), ticks (*Argas persicus*), and hippoboscids (*Ornithoica vicina*) were removed from various adult and subadult magpies. This tick appears to be a new record for Colorado. Simuliids were abundant in the streams near the nesting areas.

REFERENCES

BENNETT, G. F. AND FALLIS, A. M. 1959 Blood parasites of birds in Algonquin Park, Canada, and a discussion of their transmission. J. Parasit. **45** (supp.): 33.

COATNEY, G. R. AND JELLISON, W. L. 1940 Some blood parasites from Montana birds. J. Parasit. **26**: 158–160.

HAGEN, A. F. AND OLSEN, O. W. 1957 Species and prevalence of parasites in the blood of the American magpie (*Pica pica hudsonia* (Sabine)) in northern Colorado. Proc. Helm. Soc. Wash. **24**: 61–62.

HANSON, H. C., LEVINE, N. D. AND KANTOR, S. 1956 Filariae in a wintering flock of Canada geese. J. Wildl. Mgt. 20: 89–92.

HERMAN, C. M. 1938 The relative incidence of blood protozoa in some birds from Cape Cod. Trans. Am. Micr. Soc. **57**: 132–141.

HERMAN, C. M., REEVES, W. C., MCCLURE, H. E., FRENCH, E. M AND HAMMON, W MCD. 1954 Studies on avian malaria in vectors and hosts of encephalitis in Kern County, California. I. Infections in avian hosts. Am. J. Trop Med. Hyg. **3**: 676–695.

JORDAN, H. B. 1943 Blood protozoa of birds trapped at Athens, Georgia. J. Parasit. **29**: 260–263.

LEVINE, N. D. AND HANSON, H. C. 1953 Blood parasites of the Canada goose, *Branta canadensis interior*. J. Wildl. Mgt. **17**: 185–196.

LINSDALE, J. M. 1937 The natural history of magpies. Pacific Coast Avifauna No. 25.

MANWELL, R. D. 1955a The blood protozoa of seventeen species of sparrows and other Fringillidae. J. Prot. **2**: 21–27.

———— 1955b Relative incidence of blood parasites in robins of central New York and of the high Rockies. J. Prot. **2**: 85–88.

WAGNER, E. D. 1946 Blood parasites of the magpie and English sparrow of eastern Washington. Bird-Band. **17**: 72–73.

WEHR, E. E. AND HERMAN, C. M. 1954 Age as a factor in acquisition of parasites by Canada geese. J. Wildl. Mgt. **18**: 239–247.

THE ENTOCOMMENSAL CILIATE FAUNA OF THE PINK SEA URCHIN, *ALLOCENTROTUS FRAGILIS*[*]

JACQUES BERGER AND ROBERT J. PROFANT
Departments of Zoology, University of Illinois, and University of California, Los Angeles

Although there are numerous records of ciliates inhabiting the digestive tracts of other strongylocentrotid echinoids, no information concerning those resident in *Allocentrotus fragilis* (Jackson, 1912) Mortensen, 1942, has been published. In February 1960, the senior author's attention was directed to the presence of a faunule of ciliates inhabiting this species by Dr. A. R. Moore, Hopkins Marine Station of Stanford University, Pacific Grove, California. During the summer of 1960 the gut contents of several specimens of *A. fragilis* from widely separated localities along the Pacific Coast were examined for ciliate infestations.

MATERIALS AND METHODS

The northern collection, made by the senior author, consisted of nine specimens, all of which were infected with entocommensal ciliates. Their test diameters varied from 7.0 to 8.5 cm. They were dredged July 9, 1960, in 3 hauls from the Hydah, research vessel of the Friday Harbor Laboratories, University of Washington, about 3 mi off Slip Point, Washington, in the Strait of Juan de Fuca (latitude, 48° 18.8′ N.; longitude, 124° 13.4′ W.) at depths between 105 and 118 fathoms. Numerous specimens of *Strongylocentrotus echinoides* and one specimen of *S. purpuratus* (from 118 fathoms) were collected simultaneously.

The southern collections, made by the junior author, were dredged June 27 and September 8, 1960, from the Elsie B, commercial fishing vessel owned and operated by Mr. Harold Durrah. The collections were made during two 2-hour drags per day over a sandy substrate in the Santa Barbara Channel (latitude, 34° 5 to 8′ N.; longitude, 119° 22 to 26′ W.) at depths of 120 and 125 fathoms. The specimens of both collections, 28 in all, ranged from 7.4 to 9.6 cm in test diameter. No other echinoids were collected with them. Specimens were placed in an ice chest until they could be examined in the laboratory 8 or more hours after collection.

In examining the specimens of *A. fragilis* for ciliates, the senior author employed the method described by Beers (1948), except that the entire gut was removed for inspection under the dissecting microscope. The junior author employed the method described by Lynch (1929), and made no attempt to localize the infestations within the hosts or to determine the incidence of the infestations, due to the time lag between collection and examination. In both studies, the ciliates were fixed and prepared for study by the Chatton-Lwoff silver impregnation technique as described by Corliss (1953). Specimens from the southern collection were exposed to pyridinated silver nitrate (5 drops pyridine to 10 ml 3 percent $AgNO_3$) to facilitate the examination of their nuclei. Certain specimens from this collection were fixed with marine Da Fano's solution and then were transferred to freshwater Da Fano's before staining; however this method was less reliable than that described by Corliss.

RESULTS

The intestinal contents of the northern hosts were mainly large pellets (diameter 2 to 3 mm) containing crustacean (mysid and euphasid) exoskeletal remains as well as a small amount of mud and sand in pellet form. No algal or other plant remains were found. The intestinal content of the southern specimens differed from that of the northern specimens in that bits of algae were present and there was an absence of mud pellets. In this respect the contents of the southern specimens were similar to those described for *Allocentrotus fragilis* by Booloo-tian et al (1959) and Mortensen (1943). As the nature of the ciliate infestation is dependent in part on the nutrition of the host (Berger, unpublished data), these differences in intestinal content may be significant in explaining the results given below.

The following species of entocommensal ciliates, indicated in table 1, were found in specimens from the Strait of Juan de Fuca and from the Santa Barbara Channel. Localization

Received for publication December 15, 1960.

[*] This investigation was supported in part by a N.S.F. Training Grant in Marine Zoology (No. G7045) administered by the Department of Zoology, University of Washington, and awarded to the senior author during the summer of 1960. The manuscript was written under the tenure of a predoctoral fellowship (GF-12,553) from the division of General Medical Sciences, U.S.P.H.S. Research facilities were kindly provided by Dr. R. L. Fernald, Director, Friday Harbor Laboratories of the University of Washington. The researches of the junior author were supported by U.S.P.H.S. Training Grant 2E-70.

and incidence of ciliates within 9 specimens of *A. fragilis* are given for the northern collection only. In this study, the digestive tract of the host is considered to consist of an esophagus, intestine and rectum.

It should be mentioned that the northern collection of *A. fragilis* was infected (2 of 9 specimens) with an umagillid rhabdocoele, *Syndesmus* sp. (*franciscus* ?), similar to that reported by Boolootian et al (1959), in speci-

TABLE I. *Distribution of entocommensal ciliates from* Allocentrotus fragilis.

Species of Ciliate	Northern collection			Southern collection occurrence
	Occurrence	Location*	Incidence	
Anophrys dogieli Poljansky & Golikova, 1959	+	b, c	4/9	–
Biggaria gracilis (Powers, 1933) Kahl, 1934	+	b, c	8/9	+
Cyclidium ozakii Yagiu, 1933	+	a, c	2/9	–
Cyclidium stercoris Powers, 1935	+	a, c	5/9	–
Cyclidium sp.**	+	b, c	7/9	–
Cryptochilidium caudatum Poljansky, 1951	+	a	3/9	–
Cryptochilidium sigmoides Yagiu, 1934	+	a	7/9	+
Entodiscus borealis (Hentschel, 1924) Madsen, 1931	+	a, c	9/9	+
Entorhipidium echini Lynch, 1929	+	b	7/9	+
Entorhipidium pilatum Lynch, 1929	+	b	6/9	+
Entorhipidium tenue Lynch, 1929	+	a	3/9	+
Euplotes balteatus (Dujardin, 1841) Kahl, 1932	+	a, c	4/9	+
Madsenia indomita (Madsen, 1931) Kahl, 1934	+	a, c	5/9	–
Plagiopyliella sp. (near *P. vorax* Strelkov, 1959)**	+	a	1/9	+
Plagiopyliella sp. (near *P. pacifica* Poljansky, 1951)	–			+

* Location in host : a, entire intestine ; b, posterior region of intestine ; c, rectum.
** New species to be described elsewhere by the senior author.

DISCUSSION

The absence of some hymenostome species from the southern collections of *A. fragilis* may be due to several factors. During the time between collection and examination of the southern specimens representatives of one or more species of ciliates may have perished. It is also possible that differences in the host's nutrition, as indicated by intestinal content, may play a differential role in the establishment and maintenance of a ciliate faunule. Another possibility is the lack of other species of infected echinoids which might serve as the source of the absent entocommensal ciliates. Large numbers of *Strongylocentrotus echinoides* were collected with *A. fragilis* from the Strait of Juan de Fuca. Examination of the ciliate faunules of these echinoids revealed species identical with those resident in *A. fragilis*. Thus, the ciliate fauna of *A. fragilis* of the northern collection closely resembles that described for *Strongylocentrotus* spp. by Berger (1960a, b). However, the southern collections of *A. fragilis* were unaccompanied by any other species of echinoid. Although *Lytechinus* spp. have been recovered at a depth of 40 fathoms around the Channel Islands (Clark, 1948), none have been recovered in the region where the southern collections were made.

mens from Monterey Bay, California. None were recovered in collections from the Santa Barbara Channel.

REFERENCES

BEERS, C. D. 1948 The ciliates of *Strongylocentrotus droebachiensis:* incidence, distribution in the host, and division. Biol. Bull. **94**: 99–112.

BERGER, J. 1960a Holotrich ciliates entocommensal in the sea urchin *Strongylocentrotus echinoides* from San Juan County, Washington. J. Parasit. **46**: 164.

——— 1960b The entocommensal ciliate fauna of *Strongylocentrotus* spp. from the northeast Pacific. J. Prot. **7** (supp.) : 17.

BOOLOOTIAN, R. A., GIESE, A. C., TUCKER, J. S. AND FARMANFARMAIAN, A. 1959 A contribution to the biology of a deep sea echinoid, *Allocentrotus fragilis* (Jackson). Biol. Bull. **116**: 362–372.

CLARK, H. L. 1948 A report on the Echini of the warmer Eastern Pacific, based on the collections of the *Velero III*. Allan Hancock Pacific Exped. **8**: 225–352.

CORLISS, J. O. 1953 Silver impregnation of ciliated protozoa by the Chatton-Lwoff technique. Stain Tech. **28**: 97–100.

LYNCH, J. E. 1929 Studies on the ciliates from the intestine of *Strongylocentrotus*. I. *Entorhipidium* gen. nov. Univ. Calif. Publ. Zool. **33**: 27–56.

MORTENSEN, T. 1943 A Monograph of the Echinoidea. Vol. III, pt. 3, Camarodonta II, Echinidae, Strongylocentrotidae, Parasalenidae, Echinometridae. C. A. Reitzel, Copenhagen.

OBSERVATIONS ON THE SPOROGONY AND CHROMOSOME CYCLE OF *GREGARINA POECILOCERUM**

C. C. Narasimhamurti and P. N. Ganapati
Department of Zoology, Andhra University, Waltair

A number of investigators have reported the occurrence of both gametic and zygotic meiosis in acephaline and cephaline gregarines. In the acephaline gregarines, gametic meiosis has been observed in *Monocystis rostrata* (Mulsow, 1911), *Monocystis agilis* (Bastin, 1919), *Monocystis* sp. (Calkins and Bowling, 1926), *Monocystis sp.* and *Urospora lagidis* (Naville, 1927). Zygotic meiosis has been reported for *Diplocystis schneideri* (Dobell and Jameson, 1915 and Jameson, 1920). *Monocystis mrazeki* (Hahn, 1929), *Monocystis arndti* (Valkanov, 1935), and *Apolocystis elongata* (Phillips and Mackinnon, 1946).

Among the cephaline gregarines, with the exception of *Echinomera hispida* (Schellack, 1907) and *Stenophora juli* (Trégouboff, 1914) which are reported to have gametic meiosis, all other forms, so far studied, namely, *Monoductus lunatus* (Ray and Chakravarty, 1933), *Hyalosporina cambolopsisae* (Chakravarty, 1935), *Zygosoma globosum* (Nobel, 1938, *Stylocephalus longicollis* (Grell, 1940), and *Gregarina blattarum* (Sprague, 1941) show zygotic meiosis. The conclusions arrived at by Noble (1938) on the chromosome cycle of *Zygosoma globosum*, based on the examination of a single cyst, are according to Sprague (1941) unconvincing and open to suspicion. In *Gregarina ovata*, however, both gametic (Paehler, 1904, and Schnitzler, 1905) and zygotic (Schellack, 1907) meiosis have been reported.

Jameson (1920) undertook a detailed investigation of the nuclear cycle in the gregarine *Diplocystis schneideri* and showed that the gametes were haploid containing a set of three chromosomes. Only the fertilized zygote had the full complement of six chromosomes, which in the first meiotic division again became haploid.

Grell (1940), investigating *Stylocephalus longicollis*, reviewed critically the literature on the chromosome cycle in gregarines and suggested that perhaps all gregarines have zygotic meiosis. A re-investigation, however, of those forms where gametic meiosis has been reported seems desirable; until this is done the possibility of gametic meiosis in some cannot be ruled out.

At the present state of our knowledge it is not quite clear what kind of an influence the haploid or diploid set of chromosomes exerts on the trophic phase of the parasite. Wenrich (1954), in his review "Sex in Protozoa," states: "It may make little difference whether the trophic existence of the individual is under the control of the haploid or diploid set of chromosomes."

In the present investigation, the authors have made a few additional observations on the development and sporogony of a cephaline gregarine, *Gregarina poecilocerum* (Ganapati and Mrutyunjaya Devi, 1954). The nuclear changes accompanying gametogenesis and sporogony have also been dealt with in some detail which in the main has confirmed Sprague's observations on *Gregarina blattarum* (Sprague, 1941).

MATERIAL AND METHODS

The host, the grasshopper *Poecilocerus pictus*, occurs in fairly large numbers on the Andhra University Campus feeding voraciously on *Calotropes* and *Oleander* leaves. In nature the infection is usually heavy among the hosts and, hence, it was unnecessary to infect the hosts experimentally. By keeping the insects in glass finger bowls and feeding them their natural diet, a large number of cysts can always be collected from the faecal matter. The cysts after collection were carefully washed and kept in a moist chamber, in watch glasses containing 2.5 percent solution of potassium dichromate. The cysts remained alive under the above conditions and sporulation took place normally in about 24 to 36 hours.

The cysts were removed from the moist chamber at regular intervals for preparation of smears and sections. By gently rolling them on a filter paper, the gelatinous ectocyst was removed, and the cysts were broken on a clean slide with a

Received for publication September 9, 1960.
* From a thesis by the first author approved for the award of degree Doctor of Science from the Andhra University.

pair of fine needles. The contents were allowed to spread evenly on the clean slide and fixed in a wet condition with Schaudinn's fluid, alcoholic Bouin's, corrosive sublimate-acetic, or Carnoy's fluid. The preparations were stained with Heidenhain's iron-alum haematoxylin, Feulgen, or Giemsa. Removal of the outer gelatinous ectocyst prior to fixing gave better results for preparing sections.

For observing the process of encystment, associating sporonts were transferred to fresh undiluted egg albumen or the body fluid of the host and watched under a binocular dissection microscope. Normal saline medium was found to be unsatisfactory for this purpose.

OBSERVATIONS

The morphology and life-cycle of *Gregarina poecilocerum* have been described by Ganapati and Mrutyunjaya Devi (1954). Therefore, the present account will be restricted to details of sporogony with special reference to the chromosome cycle.

Chromosome Cycle

Considerable difficulty was experienced in fixing the cysts intact and getting satisfactory sections. Many known fixatives and some modifications were tried. An ideal fixative which would not rupture the cysts in the process of fixation and which would not later interfere with sectioning was, however, not found. The present account is therefore largely based on observations of smear preparations from cysts fixed at regular intervals. The few good sections that were obtained were helpful in confirming the fact that the number of chromosomes involved in the divisions preceding gamete formation is three. The gametes produced by the two gametocytes are similar in shape and, although they appear motile when they are within the cysts, no locomotor organelle of any kind was observed either in fresh preparations examined under bright and dark-field illumination or in stained material.

The fully formed gametes are oval in shape measuring 3.5 by 2.0 microns with a vesicular nucleus placed at the narrower end (fig. 1). In later stages, the nuclei appear compact, staining homogeneously with haematoxylin and Feulgen stains. A nuclear membrane is not clearly visible at any stage even though a clear space is always present around the nucleus both in fresh and stained preparations.

Two gametes which appear identical morphologically come together and lie side by side with their nuclear ends facing each other (fig. 2). This is followed by a cytoplasmic fusion when the two nuclei occupy a common perinuclear space (fig. 3) in the zygote. We have not been able to decide whether from a physiological viewpoint one of the gametes is more active than the other as Sprague (1941) noticed in *Gregarina blattarum*.

The chromatin material in the two nuclei is seen as deeply staining tangled threads. At a slightly later stage, an increase in volume of the threads accompanied by a decrease in its intensity of staining reaction is seen (fig. 4). Two groups of three chromosomes each are ultimately formed from the nuclei (fig. 6). The chromosomes in each group are not of the same size and one of them is always longer than the other two (fig. 9). Each chromosome shows a number of chromomeres. The appearance of the chromosomes in the form of long threads with a large number of chromomeres suggests that this is the leptotene stage (figs. 6, 7). The six chromosomes, three from each gamete nucleus, come together and arrange themselves in a "Bouquet" (fig. 8).

In the next stage the chromosomes associate in pairs, the members of each pair being identical in size and shape (Fig. 9). Three tetrads, two short and one long, can be recognized in the common perinuclear space. One of the interesting features of the meiosis of *Gregarina poecilocerum* is the delayed disjunction of one of the tetrads formed by the long chromosomes. The two shorter ones separate earlier than the longer one (fig. 11). It may probably be due to delayed disjunction. We have also not been able to make out from our preparations a distinct metaphase. The staining reaction from the earlier stages to the polarized one is more or less unchanged, but after disjunction there is a notable increase in the staining reaction as a result of condensation of the chromosomes.

The shorter tetrads separate earlier and migrate to the poles while the long one is still in the center of the perinuclear space with the result that a stage with five chromatin bodies, four of which are dyads and one a tetrad, can be recognized (fig. 12). In the late anaphase the longer tetrad also separates and all the three dyads are close together. At this stage it is not possible to identify them individually (fig. 13). In the telophase, the daughter nuclei are reconstituted (fig. 14). The interphase nucleus at the end of the first division is vesicular and the

chromatin is present as three clumps adhering to the inner margin of the nuclear membrane (fig. 16). Two nucleated sporoblasts are relatively rare. It would appear that the second division follows almost immediately after the first division is completed. In the prophase of the second division the chromosomes appear as long tangled threads; it is not possible to count their number (fig. 18). The chromosomes become clumped in the next stage. In the early anaphase, however, it is possible to distinguish individual chromosomes which appear much smaller than those of the previous division (fig. 19). In the telophase, the three chromosomes are clumped, and they slowly condense and assume a rounded form (fig. 20). In the interphase, the nucleus is vesicular with the chromatin material concentrated along the periphery (fig. 21). The nucleus in some cases appears as an incomplete ring or a crescent due to the concentration of the chromatin material peripherally (fig. 22). The chromatin may also appear concentrated as a spherical, excentrically placed endosome. By a third division eight nuclei are produced which form the sporozoite nuclei (figs. 23 to 25). The nuclei are ring-shaped, and the chromatin material is distributed homogeneously along the inner margin of the nuclear membrane. The nuclei, which are at first scattered, migrate towards the center of the sporoblast. Instead of the nuclei being spherical, they may become ovoid due to an elongation.

From the account given above, it is clear that each gamete contributes three chromosomes in the formation of the synkaryon, which has the diploid number of six chromosomes. The first nuclear division of the synkaryon is meiotic; the second is equational. The third division is also mitotic, and the same haploid number continues in the sporozoite and later trophic stages of the parasite.

Sprague (1941) observed that in mature cysts the great majority of spores contained degenerating nuclei, whose chromatin appears as "scattered masses inconstant in size, number, and arrangement." The author presumed that the spores remained viable for only a very short time after maturity. No attempt, however, was made by this author to test the viability of the spores. We have not found any evidence of degeneration of the nuclei of the sporozoites, and our observations on the spores of *Gregarina poecilocerum* have shown that the spores remain viable for long periods. MacDougall (1942) has also found that the spores of *Tenebrio* gregarines remain viable for a period of 90 days at 37.5 C.

The ripe sporocyst containing eight nuclei of the sporozoites is readily stained with the Feulgen technique. In some instances, however, the spore wall was highly resistant and as such the sporocyst remained unstained.

DISCUSSION AND CONCLUSIONS

The chromosome cycle in gregarines has been studied by only a few workers and, as already pointed out in an earlier part of this paper, there has been no unanimity of view as to the exact stage in the life cycle at which the reduction division takes place. An odd number of chromosomes has been observed during gametogenesis in several gregarines. Schellack (1907) and Leger and Duboscq (1909) assumed this odd number to be diploid and suggested that it is due to an "unpaired or accessory chromosome" which they identified by its large size. In *Gregarina ovata,* which shows an odd number of three chromosomes, the odd chromosome is hardly, if at all, recognizable (Schellack, 1907). There have also been suggestions in the literature that the accessory or the axial chromosome forms the karyosome of the resting nucleus. In *Gregarina ovata,* however, no karyosome is found although it shows an odd number of chromosomes.

Jameson (1920) has conclusively shown that the odd number of chromosomes is the haploid number. Dobell and Jameson (1915), discussing the question of chromosome reduction in gregarines, referred to two cases of "alleged reduction during gametogenesis." In *Stenophora juli,* where Trégouboff (1914) reported reduction during gametogenesis, Dobell and Jameson (1915) state that a careful examination of his figures show that they are misinterpreted. Similarly, in the case of *Monocystis rostrata* (Mulsow, 1904), where reduction from eight to four chromosomes during gametogenesis has been reported, Dobell and Jameson (1915) state: "A careful examination of the work shows that Mulsow probably dealt with two different species one with eight and the other with four chromosomes."

Since then, however, a few more cases of gametic meiosis have been reported, as follows: in *Monocystic agilis* (Bastin, 1919); *Monocystis*

sp. (Calkins and Bowling, 1926); *Monocystis sp.;* and *Urospora lagidis* (Naville, 1927).

Although Dobell and Jameson (1915), Grell (1940), and Sprague (1941) believe that zygotic meiosis may occur in all gregarines, it is desirable to reexamine those gregarines which are reported to show gametic meiosis before such a generalization is made.

In the present form, we have seen clear evidence of zygotic meiosis. Only three chromosomes are present during gametogenesis, and one of the three is longer than the other two. This chromosome appears to be the "odd or accessory or axial" chromosome observed by previous workers. During the meiotic division of the synkaryon, the paired odd chromosome lags behind the other two pairs and disjunction is delayed. In the anaphase, three chromosomes move to each pole and the telophase nuclei are reconstituted. The second division is typically mitotic. Three chromosomes are clearly seen at the anaphase stage. The third division also presumably is mitotic although the chromosomes are not distinct owing to their small size.

SUMMARY

1. The chromosome cycle after gametogenesis has been traced, and it has been shown that the reduction division is zygotic. The zygote is diploid having the full complement of six chromosomes. All other stages including the gametes are haploid.
2. The present investigation supports the view that the odd number of chromosomes reported in several other gregarines may prove to be the haploid number.
3. The presence of a long chromosome has been noted. It behaves in a peculiar manner during the meiotic division of the synkaryon.
4. The sporocysts remain healthy and viable for long periods after they are liberated.

LITERATURE CITED

BASTIN, A. 1919 Contribution à l'étude des grégarines monocystidées. Bull. Biol. France et Belgique **53**: 325–373.

CALKINS, G. N. AND BOWLING, R. C. 1926 Gametic meiosis in Monocystis. Biol. Bull. **51**: 385–399.

CHAKRAVARTY, M. M. 1935 Studies on Sporozoa from Indian millipedes. IV. Life-history of a cephaline gregarine, *Hyalosporina cambolopsisae*, n. gen., n. sp. Arch. f. Prot. **86**: 211–218.

DOBELL, C. AND JAMESON, A. P. 1915 The chromosome cycle in coccidians and gregarines. Proc. Roy. Soc. Lond. **89**: (series B): 83–94.

GANAPATI, P. N. AND MRUTYUNJAYA DEVI, C. S. 1954 On the life-history of a new cephaline gregarine, *Gregarina poecilocerum* sp. nov. from the gut of the grasshopper, *Poecilocerus pictus* (*Fabr.*). Jour Zool. Soc. India **6**: 133–144.

GRELL, K. G. 1940 Der Kernphasenwechsel von *Stylocephalus* (*Stylorhynchus*) *longicollis* F. Stein. Arch. f. Protistenk. **94**: 161–200.

HAHN, J. 1929 Courte description du cycle évolutif de *Monocystis mrazeki*, ûn nouveau parasite du genre Rhynchelmis. Ann. Protist. **2**: 78–100.

JAMESON, A. P. 1920 The chromosome cycle in gregarines, with special reference to *Diplocystis schneideri* Kunstler. Quar. J. Micr. Sci. **64**: 207–266.

LÉGER, L. AND DUBOSCQ O. 1909 Études sur la sexualité chez les grégarines. Arch. f. Prot. **17**: 19–127.

MACDOUGALL, M. M. 1942 A study of the temperature effects on the gregarines of the *Tenebrio* larvae. J. Parasit. **28**: 233–241.

MULSOW, K. 1911 Über Fortpflanzungserscheinungen bei *Monocystis rosirata* n. sp. Arch f. Prot. **22**: 20–55.

NAVILLE, A. 1827a Le cycle chromosomique d' *Urospora lagidis* (De Saint Joseph). Parasitol. **19**: 100–138.

————. 1927a Le cycle chromosomique et la meiose chez les *Monocystis*. Z. Wiss. Biol. B. Zeitschr. Zellforsch. milkroskop. Anat. **6**: 257–284.

NOBLE, E. R. 1938 The life-cycle of *Zygosoma globosum* sp. nov., a gregarine parasite of *Urechis caupo*. Univ. Calif. Publ. Zool. **43**: 41–66.

PAEHLER, F. 1904 Über die Morphologie, Fortpfanzung und Entwicklung von *Gregarina ovata*. Arch. f. Prot. **4**: 64–87.

PHILLIPS, N. E. AND MACKINNON, D. L. 1946 Observations on a monocystid gregarine, *Apolocystis elongata* n. sp. in the seminal vesicles of *Eisena foetida*. Parasitol, **37**: 65–74.

RAY, H. N. AND CHAKRAVARTY, M. M. 1933 Studies on Sporozoa from Indian millipedes. II. The life-history of a cephaline gregarine, *Monoductus lunatus* n. gen., n. sp. Arch. f. Prot. **81**: 352–360.

SCHELLACK, C. 1907 Über die Entwicklung und Fortpflanzung von *Echinomera hispida*. Arch. f. Prot. **9**: 297–345.

SCHNITZLER, H. 1905 Über die Fortpflazung von *Clepsidrina ovata*. Arch. f. Prot. **6**: 309–333.

SPRAGUE, V. 1941 Studies on *Gregarina blattarum* with particular reference to the chromosome cycle. Univ. Ill. Biol. Monog. **18**: 1–57.

TRÉGOUBOFF, G. 1944 Sur l' évolution sexuelle de *Stepnophora juli* A. Schneider et la position systématique de la famille des Stenophoridés Léger et Duboscq. Arch. Zool Exp. et Gen. **54**: (Notes et Rev.): 18–29.

VALKANOV, A. 1935 Untersuchungen über den Entwicklungskreis eines Turbellarienparasiten *Monocystella arndti*. Ztschr. Parasitenk. 7: 517–538.

WENRICH, D. H. 1954 Sex in Microorganisms. Am. As. Adv. Sci., Washington, D. C.

PLATE I

EXPLANATION OF PLATE I

FIGURE 1. Gamete from section (C–F).

FIGURE 2. Union of two gametes (C–F).

FIGURE 3. Zygote, the two nuclei lying in a common perinuclear vesicle (C–G).

FIGURES 4–5. Nuclei in preparation for meiotic division (C–G).

FIGURES 6–8. Leptotene. The chromosomes make their appearance and they appear polarized in Fig. 8 (C–G).

FIGURE 9. Zygotene. Homologues come in close contact. Note one of them is larger than the other two (C–G).

FIGURE 10. Pachytene, early state (C–G).

FIGURE 11. Diplotene. The long chromosomes with chiasma, one at either end. The central area appears as a loop (C–G).

FIGURE 12. Note delayed disjunction of the longer tetrad (C–G).

FIGURES 13–14. Telophase of 1st division (C–G).

FIGURE 15. Nuclear reconstitution at the end of 1st division (Ab–I.H.).

FIGURES 16–17. Sporoblast with two ring shaped nuclei (Ab–I.H.).

FIGURE 18. Prophase of the second division (Ab–I.H.).

FIGURE 19. Anaphase of the second division (C–F).

FIGURE 20. Telophase of the second division (C–F).

FIGURE 21. Sporoblast with four nuclei showing ring-shaped structure.

FIGURE 22. Chromatin concentrated in the form of an endosome which is excentrically placed (Ab–I.H.).

FIGURE 23. Prophase of the third division (C–F).

FIGURE 24. Telophase of the third division (C–G).

FIGURE 25. Eight ring-shaped nuclei in the sporoblast (C–F).

Ab, alcoholic bouins; C, Carnoy; F, Feulgen; G, Giemsa; I.H., iron haematoxylin.

PLATE I

EFFECT OF SALICYLATES AND RELATED COMPOUNDS ON ABLASTIC RESPONSE IN RATS INFECTED WITH *TRYPANOSOMA LEWISI*. I. ISOMERS OF SALICYLIC ACID*

ELERY R. BECKER**

Division of Life Sciences, Arizona State University, Tempe

Trypanosoma lewisi reproduces asexually for a few days after introduction into the blood of susceptible rats, after which it ceases to multiply, its population becomes reduced by trypanolytic antibody, and the survivors assume the "resting" or "adult" form. The dynamics for the transition of the population from the reproducing to the "resting" phase are believed to consist of host responses to the parasitemia which culminate in the formation of the circulating reproduction-inhibiting antibody that Taliaferro (1932) named ablastin. The transition from reproducing to adult population has been prevented by the administration of sodium salicylate through a stomach tube (Becker and Gallagher, 1947). Later Barnes (1951) extended the study so that not only sodium salicylate, but also salicylic acid, methyl salicylate, acetylsalicylic acid (aspirn), and phenyl salicylate were shown to be capable of producing the antiablaste effect.

The present study concerns the effect of two isomers of salcylic acid, viz., meta-hydroxybenzoic acid and para-hydroxybenzoic acid, on what is here called the ablastic response. The positions of the hydroxyl group on the benzene ring in ortho-hydroxy-, meta-hydroxy-, and para-hydroxybenzoic acid are shown in figure 1.

MATERIALS AND METHODS

The strain of *T. lewisi* is believed to be the same as that previously employed, which stems from a naturally occurring laboratory infection (see Becker, Manresa and Johnson, 1943). The host was the Sprague-Dawley strain of white rat. All the rats in a particular experimental series were from either one litter or two of approximately the same age. The weights varied from 160 to 280 g.

There were two controls in each experiment except for series 5: Untreated infected rats and infected rats treated with salicylic (ortho-hydroxybenzoic) acid. Because of their low solubility in distilled water, salicylic acid, meta-hydroxybenzoic acid, and para-hydroxybenzoic powders were dissolved in a solution of 3 percent sodium bicarbonate with the aid of stirring. As in our previous experiments, drug solutions were injected directly into the stomach through a small soft rubber catheter attached to a 5-cc Luer syringe. The rats were fed "Purina Laboratory Chow" supplemented occasionally with milk, to which a small amount of corn oil had been added, carrots, and lettuce.

The ortho-hydroxybenzoic (salicylic acid) and para-hydroxybenzoic acids were products of Eastman Organic Chemicals, Rochester, N. Y.; the meta-hydroxybenzoic acid was obtained from K and K Laboratories, Jamaica, N. Y.

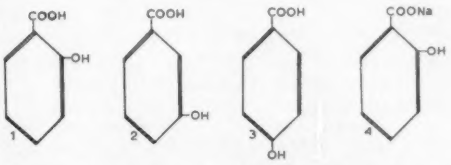


FIGURE 1. Isomers of hydroxybenzoic acid: 1, ortho; 2, meta; 3, para. 4, ortho's sodium salt.

The possible effect of sodium bicarbonate on the anti-ablastic action of salicylic acid was tested. Since the salicylic acid so treated continued to affect the ablastic response, it was assumed that it was safe to employ the same method for solubilizing the meta and para isomers of salicylic acid. All drug administrations were started at the end of 48 hours and continued daily as described. The three isomers were also administered in partial suspension after the attempt had been made to solubilize them at 50 C.

The other procedures employed have been previously described (cf. Becker, Taylor and Fuhrmeister, 1947). The coefficient of variation of length measurements of trypanosomes was employed as the index to the intensity of reproduction occurring on a particular day (cf. Taliaferro and Taliaferro, 1922). It should be explained that a value of 6 or 7 percent or lower indicates no reproduction, while higher ones indicate the occurrence of acitve reproduction.

Received for publication December 5, 1960.

* This investigation was supported in part by Research Grant E-2975 from the National Institutes of Health, Public Health Service.

** Present address: Communicable Disease Center, Phoenix, Ariz.

RESULTS

Tests for effect of sodium bicarbonate. Three rats were administered distilled water; three, 3 percent sodium bicarbonate solution; three, 5 percent sodium salicylate dissolved in distilled water; and three, 5 percent salacylic acid dissolved in 3 percent sodium bicarbonate solution, all at a level of 1 cc per 100 g body weight commencing 48 hours after inoculation and thereafter daily for 8 days. The recipients of distilled water and of sodium bicarbonate solution developed typical infections with complete cessation of reproduction after 7 to 9 days. The recipients of sodium salicylate and of salicylic acid dissolved in 3 percent sodium bicarbonate were still reproducing actively at the end of 10 days, and all had died after 12 days with tremendous trypanosomal parasitemias. Inspection of the stained blood smears throughout the course of the infections revealed little or no differences in the parasitemias in the six salicylate recipients.

Tests for effect of m- and p-hydroxybenzoic acids. The data presented in table I cover three series of experiments in which the drugs were dissolved in 3 percent sodium bicarbonate. In another series, not shown in the table, they were partially dissolved in warm (50 C) distilled water and partially suspended (series 4). There were in all 9 control rats which received only distilled water, 12 controls which received dosages of ortho-hydroxybenzoic acid sufficient to demonstrate the effectiveness of that drug in producing the anti-ablastic effect, 14 recipients of m-hydroxybenzoic acid and 14 of p-hydroxybenzoic acid.

TABLE I. Coefficient of variation for lengths of individual trypanosomes and thousands of trypanosomes per cubic millimeter of blood (in parenthesis) on selected days of infections in rats treated with hydroxybenzoic acids; daily dosages in parenthesis under drug designation are for 100 g rat body weight. (Abbreviations: OHBA, ortho-hydroxybenzoic acid; MHBA, meta-hydroxybenzoic acid; PHBA, para-hydroxybenzoic acid; D, rat dead; — (dash), no data; M, male; F, female.)

Series	Rat No.	Treatment (dose)	Days after inoculation 5	7–8	10	12	17	20
	1M	H_2O	25 (38)	21 (242)	17 (288)	7 (220)	5 (160)	— (2)
	2F	"	14 (40)	24 (140)	19 (400)	16 (84)	5 (+)	— (0)
	3M	OHBA (50 mg)	20 (190)	23 (740)	27 (980)	19 (560)	6 (80)	5 (10)
1*	4M	MHBA (50 mg)	17 (60)	21 (148)	9 (344)	10 (201)	9 (160)	8 (62)
	5F	"	24 (80)	23 (400)	18 (760)	8 (314)	7 (206)	6 (48)
	6M	PHBA (50 mg)	30 (30)	25 (170)	12 (400)	9 (270)	8 (72)	5 (17)
	7F	"	10 (66)	18 (225)	9 (490)	10 (240)	8 (81)	6 (16)
	8M	H_2O	29 (72)	11 (680)	9 (49)	6 (58)	6 (32)	5 (29)
	9F	"	19 (33)	10 (290)	7 (131)	5 (72)	6 (66)	4 (14)
	10M	OHBA (55 mg)	20 (720)	20 (1010)	28 (600)	17 (310)	6 (32)	6 (9)
	11F	"	24 (780)	26 (970)	20 (1220)	14 (260)	6 (90)	5 (5)
2*	12M	MHBA (55 mg)	20 (24)	11 (42)	7 (35)	4 (36)	6 (11)	5 (8)
	13F	"	28 (800)	16 (740)	10 (480)	6 (88)	5 (22)	4 (0)
	14M	PHBA (55 mg)	19 (16)	11 (34)	8 (29)	4 (17)	5 (6)	6 (0)
	15F	"	32 (62)	12 (42)	9 (40)	7 (20)	4 (20)	6 (8)
	16F	H_2O	25 (740)	21 (745)	11 (241)	6 (170)	5 (41)	— (0)
	17F	"	14 (66)	24 (110)	10 (85)	5 (44)	6 (16)	5 (3)
	18F	OHBA (60 mg)	19 (541)	26 (1020)	22 (730)	28 (841)	D	
3**	19M	MHBA (60 mg)	17 (116)	21 (440)	9 (216)	9 (166)	7 (47)	6 (8)
	20F	"	24 (84)	23 (230)	14 (260)	7 (145)	8 (44)	7 (17)
	21M	PHBA (60 mg)	30 (685)	25 (410)	12 (205)	9 (108)	8 (71)	5 (32)
	22F	"	10 (66)	17 (81)	9 (77)	11 (42)	7 (30)	4 (16)

* Drug dissolved in soda solution, administered days 2 to 7.
** Drug dissolved in soda solution, administered days 2 to 8.

Inspection of the table shows that undrugged controls responded in the classic manner with low coefficients of variation, indicating no reproduction of the trypanosomes on or after 10 days following inoculation. Treatment with the ortho drug in dosages of 50 to 60 mg per 100 g rat weight commencing 2 days after inoculation and continuing thereafter for another 5 to 7 days resulted in reproducing trypanosome populations with high size (length) variability and microscopically demonstrable division forms as well as high parasitemias, on the 12th day; one of the five hosts had succumbed with high parasitemia by thet 17th day. Inspection also reveals that the administration of the meta and para forms of the drug in the same dosages as the ortho form did not result in high size variability continuing through the 12th day. In the case of rats 4, 5, 6, 7, 19, 20, 21, and 22, however, for some reason the coefficients of variation were slightly higher than in the undrugged controls on the 10th and 12th days, but microscopic examination of the stained films revealed the presence of only "resting" or "adult" trypanosomes. It is possible that these two drugs may have had the effect of retarding somewhat the return to size uniformity which normally follows the cessation of the trypanosomes' multiplication; but such an effect, if any, is of no significance to the problem of the effect of the drugs on reproduction. The results of series 4, with its three H_2O controls, three recipients of the ortho drug, three of the meta and three of the para drug, were comparable to those of series 1, 2, and 3 shown in table I. The recipients of the ortho drug had died by the 15th day with extremely heavy parasitemias. The dosages of drugs were like those in series 1, and administration was on days 2 to 9.

Another experiment (series 5) was performed in which five rats received 45 mg of salicylic acid per 100 g of rat weight, five 45 mg of the meta isomer, and five 45 mg of the para isomer on days 2 to 8. There was no control group of water-recipients, but the results of the experiment otherwise paralleled those obtained in the four series already described in that the ortho-drug recipients exhibited inhibition of ablastic action, while the meta- and para-drug recipients supported infections whose reproductive phase terminated by the 10th day.

ACKNOWLEDGMENTS

The strain of *T. lewisi* used in these experiments, one used previously by the author, was obtained from Dr. Michael Lysenko of the University of Wisconsin who had maintained it in his laboratory for a number of years. The technical assistance of the following undergraduate students is gratefully acknowledged: Mr. Gerath A. Wilson, Mr. John Ditmar, Mrs. Rosemary Schwendt, Miss Virginia Covington, and Mr. Ernie Benesch.

SUMMARY

It is again confirmed that sodium salicylate and salicylic acid interfere with ablastin in its classic role of inhibiting reproduction of *T. lewisi*, only in this study the salicylates were dissolved in 3 percent sodium bicarbonate solution. Meta-hydroxybenzoic acid and para-hydroxybenzoic acid solubilized in 3 percent sodium bicarbonate do not produce the anti-ablastic effect domonstrated by the ortho-hydroxybenzoic acid and its sodium salt. Three percent sodium bicarbonate solution alone does not affect the intensity of reproduction or the length of the reproductive phase.

LITERATURE CITED

BARNES, E. A. 1951 Effect of benzene and six selected salicylates on the development of immunity in *Trypanosoma lewisi* infections. Iowa State Coll. J. Sci. **25**: 150–152.

BECKER, E. R. AND GALLAGHER, P. L. 1947 Prolongment of the reproductive phase of *Trypanosoma lewisi* by the administration of sodium salicylate. Iowa State Coll. J. Sci. **21**: 351–362.

———, MANRESA, M. AND JOHNSON, E. M. 1943 Reduction in the efficiency of ablastic action in *Trypanosoma lewisi* infection by withholding pantothenic acid from the host's diet. Iowa State Coll. J. Sci. **17**: 431–441.

———, TAYLOR, J. AND FUHRMEISTER, C. 1947 Effect of pantothenate deficiency on *Trypanosoma lewisi* infection in the rat. Iowa State Coll. J. Sci. **21**: 237–243.

TALIAFERRO, W. H. 1932 Trypanocidal and reproduction-inhibiting antibodies to *Trypanosoma lewisi* in rats and rabbits. Am. J. Hyg. **16**: 32–84.

——— AND TALIAFERRO, L. G. 1922 The resistance of different hosts to experimental trypanosome infections, with especial reference to a new method of measuring this resistance. Am. J. Hyg. **2**: 264–317.

RESEARCH NOTE

EMERGENCE OF CERCARIAE OF *SCHISTOSOMA MANSONI* FROM *AUSTRALORBIS GLABRATUS.*

Investigations on the relationship of *Schistosoma mansoni* to the intermediate host snail *Australorbis glabratus* have included repeated observations of the emergence of cercariae, a subject needing clarification. This paper reports studies on cercarial emergence from numerous naturally infected *A. glabratus* collected in the field as well as snails reared and infected in the laboratory.

Snails were observed alive, left side up in syracuse dishes with water shallow enough to prevent their turning over. A binocular, stereoscopic, dissecting microscope was used with magnifications of 9X, 27X, and 54X. Albino *A. glabratus* and local strains with very little black pigment were exposed to miracidia when only a few days old. Reared in the laboratory these provided specimens shedding cercariae at about 10 mm diameter, with transparent shells and almost transparent bodies.

Cercariae were observed emerging from sporocysts in the digestive gland and migrating by wriggling and twirling movements around the central sides of the whorls in the hemoglobin-tinted blood sinuses. In the heart region cercariae could be seen within the pericardium and in the aorta moving in spurts, aided by the pumping of the heart. No cercariae were seen leaving the blood system before reaching the apertural region. After emergence cercariae occasionally entered the pulmonary cavity temporarily before leaving a snail.

Cercariae were observed to emerge from the blood vessels of either the pseudobranch or the mantle edge. Usually cercariae first appeared in the vessels of the pseudobranch from which they emerged either by piercing the tissues and wriggling free or by passing along the vessel to the portion of mantle covering the callus where emergence occurred. When several cercariae were present, they could be seen lined up in a series in the blood vessels. In some cases, cercariae were observed to move back and forth in the vessel between the mantle and the pseudobranch.

When numbers of cercariae were present in the vessels of the pseudobranch or mantle, they often emerged in rapid succession through the same point in the tissues. Also, cercariae were observed to emerge from the vessels of the mantle collar.

Emergence of three kinds of cercariae other than *S. mansoni* from *A. glabratus* and five kinds from other snails (*Tropicorbis riisei, Drepanotrema anatinum, Lymnaea columella,* and *Plesiophysa*) was observed. In all of these, emergence was similar to that of *S. mansoni* cercariae from the vessels of the pseudobranch and mantle margin.

The observations here reported corroborate those of Duke (1952, J. Helminth, **26**: 133–146).

The earliest emergence of cercariae was observed on the 17th day after exposure of a laboratory-reared. *A. glabratus* to miracidia of *S. mansoni.* Laboratory temperatures ranged from 78° F to 86° F. In a number of snails, emergence was observed on the 18th day, which was the first day of observation for them. Emergence of *S. mansoni* cercariae from *A. glabratus* in 18 days was reported by Stirewalt (1954, Exp. Parasit. **3**: 504–516). CHARLES S. RICHARDS, *Puerto Rico Field Station, Technical Development Laboratories, Technology Branch, Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, San Juan, Puerto Rico.*

ON THE OCCURRENCE OF *TRICHINELLA SPIRALIS* IN PORK SAUSAGE AVAILABLE IN IOWA (1953–60)*

W. J. Zimmermann, L. H. Schwarte and H. E. Biester
Veterinary Medical Research Institute,
Iowa State University of Science and Technology, Ames

Commercial pork sausage available in Iowa has been examined for the presence of *Trichinella spiralis* larvae during the periods 1944–46 and 1953–60. A preliminary report by Zimmermann, Schwarte and Biester (1956) had shown prevalence of trichinae in fresh bulk and fresh link pork sausage during 1944–6 of 12.4 percent and 11.7 percent, respectively, while studies during the 1953–55 period indicated a significant decrease to 1.2 percent in bulk and 3.4 percent in link sausage. Studies have been continued through June 1960, to determine if a further decrease had occurred or if the lower prevalence levels would be maintained during the period in question.

Fresh pork sausage, available in both bulk and link type, is not processed to kill trichinae. Salt and spices are added for flavor only. Pork products, which are generally considered ready to eat when produced under the Federal Meat Inspection, are processed by the manufacturer to destroy the vitality of any *T. spiralis* larvae which may be present in the pork. The United States Department of Agriculture, Meat Inspection Division (1960) has prescribed regulations which are sufficient to destroy any trichinae present. These regulations include heating to a temperature of 137 F or higher throughout all parts of the pork product, refrigeration at 5 F, –10 F or –20 F for specified periods, and special curing methods which include the addition of specified amounts of salt prior to regulated smoking and drying procedures.

The prevalence of *T. spiralis* in commercial pork sausage of types not processed to destroy trichinae has varied in different areas of the country. Dickman (1938) obtained trichinae from 6 to 63 samples from Philadelphia. Peres (1942) examined 50 samples of sausage in New Orleans and found all negative. Kerr (1942) in California, found 21 of 1056 samples to contain trichinae. The 21 samples contained 1166 larvae of which 121 were classified as dead. Four of the samples contained only dead larvae. Kerr also examined 609 samples of various types of pork products which were processed to kill trichinae. None of the 609 products contained either living or dead larvae. Schwartz (1939) examined 1118 half-pound samples of federally inspected frankfurters. Only 11 contained trichinae, with a maximum of 2 larvae being recovered. In no case did the worms show evidence of viability. Harrington, Spindler and Hill (1950) reported the examination during 1934–39 of 13,013 half-pound samples of pork products which were treated to kill trichinae. Trichinae were recovered from 433 (3.32 percent). Only 4 contained living larvae, all of which were adjudged to be non-viable. The maximum larval count was 2400. Similar studies during 1948–49 found only 26 (0.82 percent) of 3171 samples to contain trichinae, with 24 containing only dead larvae. The maximum larval count was 120.

MATERIALS AND METHODS

During the 7-year period July 1953 through June 1960, 8402 fresh bulk sausage samples, 1432 fresh link sausage samples and 861 samples of processed link pork sausage treated by curing and/or heat to kill trichinae were examined for the presence of *T. spiralis*. All samples were obtained from retail sources. Examination of the sausage was made by the artificial digestion-Baermann technique of Kerr, Jacobs and Cuvillier (1941).

A 45-g (0.1-lb) sausage sample was placed in a 3-liter beaker to which 2700 ml of a stock solution of 0.5 percent pepsin and 0.7 percent concentrated hydrochloric acid was added. The material, maintained in an incubator at 37 C, underwent constant mechanical agitation with plastic paddles for 18 hours. The paddles were then removed and the sediment allowed to settle for 1 hour. Then two-thirds of the supernatant fluid was siphoned off. The re-

Received for publication January 12, 1961.

* Supported in part by a research grant, E606(C5), from the National Institutes of Health, U. S. Public Health Service, Published as paper N.S. 613, Veterinary Medical Research Institute, College of Veterinary Medicine, Iowa State University of Science and Technology, Ames, Iowa.

mainder of the digested material was poured into the Baermann apparatus, consisting of an 80-mesh screen fitted into the top of a 3-liter funnel. Water at 37 C was then added to cover the screen. The mixture was allowed to stand for at least 1 hour after which approximately 500 ml was drawn into a smaller funnel. This was again allowed to stand for at least 1 hour. Then fluid was drawn into a ruled Syracuse watch glass for microscopic examination at 27 ×. Examination was made immediately, since living trichinae will normally be moving in warm liquid. When the liquid cools, the trichinae normally will coil. When trichinae were found, draws were made from both small and large funnels until trichinae were no longer recovered.

No attempt was made to determine the viability of trichinae recovered. Future studies will include feeding of recovered trichinae to experimental animals to verify viability. This should be especially valuable for trichinae recovered from sausage processed to kill trichinae since the worms recovered may have been devitalized by the processing technique.

RESULTS

The studies on the occurrence of *T. spiralis* in commercial pork sausage available in Iowa have shown that there has been a marked change in prevalence of trichinae. Studies during the 1944–46 period had shown an incidence of 12.4 percent in the 460 samples of bulk pork sausage examined. This was much higher than that obtained during the present series of studies (table I). During the 7-year period 1953–60, trichinae were present in only 1.0 percent of the 8402 samples of bulk pork sausage examined. Although the yearly incidences tended to fluctuate, there was a more or less continual decrease in occurrence. The prevalence in bulk sausage during 1953–54 was 3.1 percent which in considerably higher than the 0.3 percent obtained during the 1959–60 period.

TABLE I. *Pork sausage samples positive for* T. spiralis.

	Bulk			Link			Processed link		
	Samples	No.	Pct.	Samples	No.	Pct.	Samples	No.	Pct.
1944–46	460	57	12.4	444	52	11.7			
1953–54	359	11	3.1	214	5	2.4	176	0	
1954–55	1093	7	0.6	373	15	4.0	487	0	
1955–56	666	15	2.3	181	5	2.7	77	0	
1956–57	1447	20	1.4	250	3	1.2			
1957–58	1368	4	0.3	145	1	0.6	56	0	
1958–59	1582	25	1.5	113	3	2.6	42	2	4.7
1959–60	1887	6	0.3	156	2	1.3	23	0	
Total 1953–60	8402	88	1.0	1432	34	2.4	861	2	0.2

Somewhat similar results were noted for the occurrence of trichinae in fresh link pork sausage. Examination of 444 link sausage samples during the 1944–46 period had revealed an incidence of 11.7 percent while in the present series, only 2.4 percent of 1432 link sausage samples contained trichinae. The more or less continual downward trend noted for bulk sausage during the 1953–60 period was not as evident for link sausage. The incidences varied from a maximum of 4.0 percent in 1954–55 to 0.6 percent in 1957–58. During the 1959–60 period trichinae were found in 1.3 percent of the samples examined.

A new type of precooked link sausage was developed in the interval between the 1944–46 and 1953–60 series which is available in both smoked and nonsmoked varieties. Trichinae were recovered from only 2 of 861 samples of processed (smoked or heat-treated) link examined during the 1953–60 period. The two positive precooked samples contained 20 and 1 larvae respectively. Thirteen of the 20 larvae in the first sample were dead, as was the single larva in the second. No determination was made of actual ability to infect.

The number of trichinae found in the 45-g (0.1-lb) samples of positive sausage is shown in table II. Eighty-nine of the 124 positive samples of the 3 types of sausage contained 5 or fewer parasites. The average trichinae recovery was 12.6 from bulk and 19.5 from fresh link. Three bulk samples contained more than 50 trichinae, the maximum being 244. The maxi-

TABLE II. T. spiralis *in positive sausage samples (1953–60).*

Worms per 45 g sample	Bulk	Link	
		Fresh	Processed
1– 5	68	20	1
6–10	9	5	0
11–15	3	3	0
16–20	3	1	1
21–30	1	2	0
31–50	1	0	0
Over 50	3	3	0
Mean number per positive sample	12.6	19.5	10.5

mum number of larvae recovered from a link sample was 314 while another contained 289.

The distribution of samples containing *T. spiralis* by brands is shown in table III. Brands A-C were from retail sources and contained pork from a variety of packers. Brands D-G were from Iowa packers not federally inspected. The remainder were from federally inspected plants.

TABLE III. *Distribution of positive pork sausage samples by brands (1953–60).*

Brand	Bulk			Fresh link		
	Samples	No.	Pct.	Samples	No.	Pct.
A	5579	52	0.9	465	8	1.5
B	168	0	0	26	0	0
C	235	1	0.4	14	0	0
D	0	0	0	25	0	0
E	0	0	0	22	0	0
F	256	2	0.8	66	0	0
G	23	0	0	0	0	0
H	361	9	2.5	39	0	0
I	0	0	0	1	0	0
J	0	0	0	37	0	0
K	22	0	0	4	0	0
L	237	1	0.4	110	1	0.9
M	21	0	0	16	0	0
N	7	0	0	101	0	0
O	104	2	1.9	43	2	4.6
P	457	3	0.7	173	0	0
Q	449	12	2.7	266	11	4.8
R	12	0	0	0	0	0
S	12	2	16.7	62	12	19.3
T	5	0	0	2	0	0
U	10	1	10.0	0	0	0
V	1	0	0	0	0	0
W	17	0	0	0	0	0
X	336	2	0.5	0	0	0
Y	90	1	1.1	0	0	0
	8402	88	1.0	1432	34	2.4

Trichina larvae were found in 12 of the 21 brand types of bulk pork sausage examined. The maximum incidence was 16.7 percent in brand S. Ten percent of brand U contained trichinae while 4 other brands had an incidence of over 1.0 percent. Only 5 of 18 brands of fresh link contained *T. spiralis* larvae. Brand S again had the highest incidence, 19.3 percent containing trichinae. The prevalence of trichinae in brand Q was 4.8 percent while that for brand O was 4.6 percent. The incidence in bulk sausage for these brands was 2.7 and 1.9 percent respectively. Two processed link samples of Brand O were found to contain trichinae.

DISCUSSION

The results of these studies indicate that progress is being made toward decreasing the frequency of *T. spiralis* as a human health hazard. This should not warrant a decrease in efforts to combat this problem, however, as the hazard will exist as long as even a low prevalence of *T. spiralis* is maintained in swine.

The number of larvae recovered from the trichinae-containing pork sausage samples was relatively small. It should be remembered, however, that the pork used in making sausage is generally a pool from numerous swine. The meat from a single infected pig may be mixed with that from several trichina-free pigs giving a relatively higher percentage of trichina-containing sausage samples with a low number of trichinae per sample. Pork cuts from an infected pig would contain a larger number of trichinae than sausage made from pork from an infected pig and a number of trichina-free pigs; therefore, pork cuts could be a more important source of clinical trichiniasis in humans.

The marked decrease in the prevalence of *T. spiralis* larvae since 1944 is probably due to several factors. The passage of garbage feeding regulations in 47 of 48 states during 1953-55 is an important factor. These regulations were not passed because trichiniasis was a human health hazard, but were created primarily to eradicate a nationwide outbreak of vesicular exanthema. A report of the U. S. Department of Agriculture Animal Disease Eradication Service (1960) indicated that in July 1960, raw garbage was fed to 21,935 swine in 25 states. Cooked garbage was fed in all states to slightly over 1,000,000 swine. Only 14 of 48 states reported 100 percent monthly inspection of garbage feeding installations.

Another factor would be better swine management methods. Most of the swine in the corn belt area of the United States are grain-fed with little, if any, access to table scraps or garbage. A third factor contributing to the decrease of *T. spiralis* in pork would be widespread use of locker plants and home freezers since freezing at specified temperature-time combinations will destroy trichina larvae. The decrease in home processing of pork is also a contributing factor. Many farmers formerly had smoke houses where the processing technique was not sufficient to kill trichinae. Now most pork is cured in packing plants or locker plants using more effective techniques.

The efficacy of the regulations of the Federal Meat Inspection Service governing the prescribed treatment of pork and pork products for destruction of trichinae was evidenced by a comparison of prevalence of *T. spiralis* in fresh and processed sausage. The prevalence of 1.0 percent in fresh bulk and 2.4 percent in fresh

link was considerably higher than the 0.2 percent in link processed to kill trichinae. Although dead larvae may have been present in both processed and fresh sausage the digestive process utilized, in combination with the Baermann screen, limited their detection. Hall and Collins (1937) stated that the Baermann technique was highly effective for detecting live trichinae, but was of little value for the detection of dead trichinae. The efficiency of the Baermann apparatus depends on the movement of live trichinae through the screen. Dead worms would not be detected unless a sufficient number were present to insure that some would pass directly through the screen. Kerr, Jacobs and Cuvillier (1941) determined the efficiency of a similar artificial digestion-Baermann method for detecting dead trichinae in human diaphragms. They examined 224 diaphragms in which only dead larvae were present as determined by microscopic and artificial digestion-Baermann technique. Only 45 (20.1 percent) of the infected diaphragms were detected by the latter technique.

SUMMARY

Studies were conducted during the 1953-60 period to determine the prevalence of *T. spiralis* larvae in commercial pork sausage available in Iowa. Trichinae have been found in 1.0 percent of 8402 fresh bulk sausage samples, 2.4 percent of 1432 fresh link samples and 0.2 percent of 861 processed link samples. This is a marked decrease from studies in 1944-46 when the prevalence for bulk and nonprocessed link pork sausage was 12.4 percent and 11.7 percent respectively. The decline in prevalence is related to garbage feeding regulations, better swine management, widespread use of home freezers and lockers, and decrease in home processing of meat.

REFERENCES

DICKMAN, A. 1938 Trichinosis: Distribution of *Trichinella spiralis* in pork products sold in Philadelphia. J. Lab. & Clin. Med. **23**: 671–680.

HALL, M. C. AND COLLINS, B. J. 1937 Studies on trichinosis. I. The incidence of trichinosis by postmortem examinations of 300 diaphragms. Pub. Hlth. Rept. **52**: 468–490.

HARRINGTON, R. F., SPINDLER, L. A. AND HILL, C. H. 1950 Freedom from viable trichinae of pork products prepared to be eaten without cooking under federal meat inspection. Proc. Helm. Soc. Wash. **17**: 90–91.

KERR, K. B. 1942 Trichinosis problem in California. Report of survey, 1940–41. Sacramento, Calif., State of Calif. Dept. Pub. Hlth.

KERR, K. B., JACOBS, L. AND CUVILLIER, E. 1941 Studies on trichinosis. XIII. The incidence of human infection with trichinae as indicated by post-mortem examination of 3000 diaphragms from Washington, D. C., and 5 eastern seaboard cities. Pub. Hlth. Rept. **56**: 836–855.

PERES, C. E. 1942 *Trichinella spiralis*. II. Incidence of infection in hogs and rats in the New Orleans area. J. Parasit. **28**: 223–226.

SCHWARTZ, B. 1939 Freedom from viable trichinae of frankfurters prepared under Federal Meat Inspection. Proc. Helm. Soc. Wash. **6**: 35–37.

UNITED STATES DEPARTMENT OF AGRICULTURE, ANIMAL DISEASE ERADICATION DIVISION 1960 National Status on Control of Garbage Feeding as of July, 1960.

UNITED STATES DEPARTMENT OF AGRICULTURE, MEAT INSPECTION DIVISION 1960 Regulations governing the meat inspection of the United States Department of Agriculture.

ZIMMERMANN, W. J., SCHWARTZ, L. H. AND BIESTER, H. E. 1956 Incidence of trichiniasis in swine, pork products and wildlife in Iowa. Am. J. Pub. Hlth. **46**: 313–319.

EFFECT OF SEVERAL NEMATODES ON AN INITIAL INFECTION OF *NEMATOSPIROIDES DUBIUS* IN MICE

G. Y. H. Liu and M. H. Ivey
Department of Microbiology, School of Medicine, University of Missouri, Columbia.

Nematospiroides dubius, a trichostrongyloid parasite of mice, was first described in detail by Baylis (1926, 1927). The life cycle of *N. dubius* was first determined by Spurlock (1943) and further elucidated by Ehrenford (1954a). Spurlock (1943), Ehrenford (1954b) and Baker (1954, 1955) have contributed information concerned with host-parasite relationships between *N. dubius* and laboratory mice.

Although this parasite is of no economic or medical importance it has many characteristics that make it a desirable form for further study. It is easily maintained in mice. It is restricted to the intestinal tissues, thus making it possible to study the intestinal phase of host reactions to parastism separate from other tissues. It was the purpose of this study to elucidate factors affecting establishment and persistence of *N. dubius* in mice and to determine the effect of superinfection with *N. dubius, Ancylostoma caninum* or *Trichinella spiralis* on an initial *N. dubius* infection. These effects were measured by determining, after superinfection, (1) total egg production (2) egg production per female (3) worms remaining after superinfection (4) percentage of eggs developing normally after superinfection.

MATERIALS AND METHODS

The strain of *N. dubius* used was obtained from Dr. N. F. Baker, School of Veterinary Medicine, University of California. The method of collecting infective larvae was described by Baker (1955). The method of infecting mice by the oral route was described by Larsh and Kent (1949).

A modified Stoll-Hausheer technique (1926) was devised for quantitative determination of eggs in mouse feces. Exactly 1.0 ml of a 0.1N NaOH solution was added to a calibrated 15-ml conical centrifuge tube. Fecal pellets, collected at random from the group of mice under study, were added to displace (approximately) 0.5 ml of the total volume and then emusified, more NaOH being added if necessary. The feces were centrifuged 1 minute at 2500 rpm and the amount of sedimented feces measured, multiplied by a factor of 15 and NaOH added to equal this figure. The fecal suspension was mixed and a 0.075-ml sample withdrawn by a Stoll pipette. A 25-mm coverslip was placed over the sample and all the eggs were counted. All egg counts were done in triplicate, averaged, and results expressed as eggs per ml of centrifuged feces.

Adult counts were made using a method described by McGee et al (1957). Quantitative checks on egg viability, following experimental stress, were made using axenic culture techniques described by Weinstein and Jones (1956).

A tuberculin syringe with a blunted 18-gauge needle was used to agitate and suspend the larvae before withdrawing a sample for oral inoculation. Preliminary experiments revealed that as the amount of agitation increased larval viability decreased, especially after 300 to 350 pumps on the syringe. This effect was equalized by stepwise selection of animals from each experimental group.

An experiment was carried out to determine the effect of varying numbers of *N. dubius* on white mice and to establish egg production patterns. Three groups of eight male mice, 8 to 12 weeks old, were infected with 50, 100, 150 larvae respectively. Egg production was checked every other day by quantitative counts. When egg production reached a plateau the mice were sacrificed and the number of *N. dubius* adults was counted and correlated with egg production.

RESULTS

As shown in figure 1, egg production began about the 10th day after infection. In group I, infected with 50 larvae, the egg pattern reached

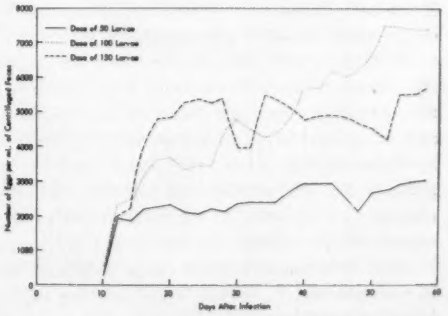


FIGURE 1. Egg production in mice infected with varying numbers of *Nematospiroides dubius*.

Received for publication April 22, 1960.

a plateau of 2,000 to 3,000 eggs per ml of centrifuged feces by the 16th day after infection. In groups II and III, infected with 100 and 150 larvae respectively, egg production was higher but much more erratic, with no definite tplateau being reached. The three groups of mice were sacrificed on the 58th day and adult counts made. As shown in table 1, group I had an average of

TABLE I. *Number of adults recovered following infection of mice with varying numbers of* Nematospiroides dubius.

Group*	Infective dose	Average number Adults recovered**		
		Female	Male	Total
I	50	27	25	52
II	100	50	42	92
III	150	62	45	107

* 8 mice per group.
** Per mouse.

52 worms; group II, of 92 worms, and group III, 107 worms. The number of eggs produced per female per ml of centrifuged feces for each of the groups was: Group I, 108; group II, 149; group III, 90. In subsequent experiments an infective dose of 50 larvae was used as it appeared to give a more consistent egg production pattern.

Four groups of eight mice each (8 to 12 weeks of age) were infected with 50 *N. dubius* larvae and egg patterns were established. The groups were then treated as follows: Group I was carried throughout the experiment as a control without any additional stress; group II was superinfected with 100 *N. dubius* larvae; group III was superinfected with 100 *A. caninum* larvae; group IV was superinfected with 100 *T. spiralis* larvae. Quantitative counts of egg production were made every other day until termination of the experiment 24 days after the superimposed infections. Also, feces were collected from each group on alternate days, the eggs concentrated, sterilized, and maintained in axenic culture media described by Weinstein and Jones (1956) and checks were made on bacterial sterility, egg viability, and percentage development. At the termination of the experiment the animals were sacrificed and worm burdens determined. Control checks indicated the *A. caninum* and *T. spiralis* larvae used for superinfection were infective.

As shown in figure 2, egg production in all four groups was around 2,000 eggs per ml of centrifuged feces before superimposition of other nematode infections. After superinfection, group I, the controls, maintained a consistent level of

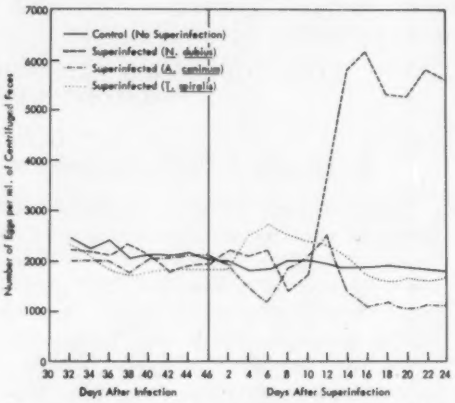


FIGURE 2. Effect of superinfection with various nematodes on an initial *Nematospiroides dubius* infection.

egg production. In group II, superinfected with *N. dubius*, the egg pattern was erratic but essentially the same for 10 days following superinfection, then a rapid increase in egg production to a peak of 6000 eggs per ml of centrifuged feces occurred. In group III, superinfected with *A. caninum*, a drop in egg production from 2000 eggs to 1000 eggs per ml of centrifuged feces was apparent. In group IV, superinfected with *T. spiralis*, egg production rose shortly after superinfection to a peak of 3000 eggs but soon returned to its original level of 1800 eggs per ml of centrifuged feces.

The four groups of mice were sacrificed 24 days after superinfection and counts of *N. dubius* adults made. As shown in table II, 41 *N. dubius*

TABLE II. *Effect of superinfection on the persistence of* Nematospiroides dubius.

Group*	Initial infection (50 larvae)	Superinfection (100 larvae)	Adult recovery (Av. per mouse)			Av. no. eggs per female**
			Female	Male	Total	
I	*N. dubius*	..	21	20	41	87
II	*N. dubius*	*N. dubius*	67	59	126	82
III	*N. dubius*	*A. caninum*	11	10	21	122
IV	*N. dubius*	*T. spiralis*	19	12	31	88

* 8 mice per group.
** Based on average egg production 16th through 24th days after superinfection.

were revealed in group I. In group II, adult counts revealed 126 worms, or about three times the number found in the control group. In group III, adult counts revealed 21 *N. dubius*. In group IV, adult counts revealed 31 *N. dubius*. The dif-

ference in adult counts between group I, the controls, and group III proved to be statistically significant at the 5 percent level.

The average number of adult female worms was correlated to the average number of eggs produced by each group during the last five quantitative egg counts as by this time (16th day through 24th day after superinfection) egg production had ceased to be erratic and had reached a plateau. From this was derived the average number of eggs produced per female worm per ml of centrifuged feces. As shown in table II, in group I, the control, a single female worm produced an average of 87 eggs; in group II, superinfected with *N. dubius,* 82 eggs; in group III, superinfected with *A. caninum,* 122 eggs; in group IV, superinfected with *T. spiralis,* 88 eggs per ml of centrifuged feces.

Observations on egg viability and development in axenic cultures revealed no differences in any of the groups.

DISCUSSION

Preliminary experiments showed that viability of larvae decreased with increasing agitation. If, however, no more than 300 to 350 pumps on a syringe are applied to a tube of larvae and animals used for infection were selected in a stepwise fashion from each group, the agitation variable did not materially affect results.

Groups of mice infected with 50, 100, and 150 *N. dubius* larvae respectively, showed different egg patterns, the pattern of the group infected with 50 larvae being more consistent. There was considerable variation in egg production per female between groups. Group I, infected with 50 larvae, produced 108 eggs per female per ml of centrifuged feces; group II, infected with 100 larvae, 149 eggs; and group III, infected with 150 larvae, 90 eggs. There was no apparent explanation for the wide group differences. Further study is indicated to determine if these differences are due to chance variation or different host-parasite relations at different intensities of infection.

Experiments conducted to determine the effect of superinfection on an initial *N. dubius* infection indicated superinfection with 100 *A. caninum* larvae caused a statistically significant (5 percent level) loss of *N. dubius* adults. One may speculate whether the loss of worms resulted from cross immunity or from a non-specific host inflammatory response directed against the *Ancylostoma* larvae, but also affecting the *N. dubius* adults. Cox (1952) reported that if *A. caninum* larvae were given to either normal mice or mice immune to *T. spiralis* 2 or 10 days before the same mice were superinfected with *T. spiralis* a premature loss of *T. spiralis* adults resulted. Goulson (1958) using the same parasites, presented similar evidence that indicated that host reactions in the anterior portion of the small intestine were primarily responsible for premature loss of *T. spiralis.* The inflammatory response in the anterior portion of the small intestine is an important factor accounting for the loss of *T. spiralis* adults in either immune or non-immune mice as shown by Larsh et al (1952) and Larsh and Race (1954). Coker (1955) using *T. spiralis* in mice suggested that cortisone reduced normal inflammatory responses in mice and in doing so masked the immune response against *T. spiralis.* Kerr (1936) presented evidence that *A. caninum* larvae caused a host inflammatory response that reaches a peak 48 hours after infection and subsequently subsides to pre-infection levels 120 hours after infection. It is conceivable that the factors elucidated from the above reported work with *T. spiralis* and *A. caninum* may hold true for the *N. dubius–A. caninum* host parasite system and that the inflammation resulting from the *A. caninum* superinfection caused the loss of *N. dubius adults.* Egg production studies revealed that the female worms in group III produced 122 eggs per female per ml of centrifuged feces versus the control's 87 eggs. Possibly the worms that survived the inflammatory response directed against *A. caninum* were more vigorous by natural selection or possibly the survivors simply had fewer brethren to compete with. However, one can not rule out the possibility that normal variation in egg sampling techniques may have caused the difference although the similarity of egg production per ml of centrifuged feces in group I (87), group II (82), and group IV (88) as shown in table II indicates little group variation occurred. This point requires further study.

Groups I, II and IV did not exhibit appreciable loss of egg production or adult worms. Group II, superinfected with *N. dubius,* showed a rise in egg production that correlated with the number of worms added to the host by superinfection. There was no apparent depression of egg production or number of worms. Similar results of a superinfection with *N. dubius* have been reported by Spurlock (1943). This phenomena is

difficult to understand in view of the considerable inflammation set up by invading *N. dubius* larvae (Baker, 1954). Also, in the case of *T. spiralis* the host inflammatory response has been shown to affect adversely the ability of adult worms to establish and persist (Larsh and Race 1954). Either quantitative and qualitative differences in inflammation are operating and/or the more superficial attachment of *N. dubius* adults as compared with *T. spiralis* affords protection from inflammatory responses. In any event, no specific immunity was directed against the second infection of *N. dubius* as applied in this experiment. Group IV, superinfected with *T. spiralis* showed a slight reduction in the number of adult worms in comparison with group I. It may be that the number of *T. spiralis* larvae used in superinfection here was not sufficient to elicit a host response detrimental to the initial *N. dubius* infection. Larsh and Race (1954) showed that peak inflammatory response of mice to an initial *T. spiralits* infection occurs on the eighth day. Perhaps the relatively long time for a peak inflammatory response to be reached (compared with *A. caninum's* 48 hours) allows the *N. dubius* adults time to adjust to adverse change in environment. The number of eggs produced per female per ml of centrifuged feces in group I (87), group II (82) and group IV (88) were practically the same.

Egg viability determinations revealed no differences between any groups as performed in these experiments.

SUMMARY

Preliminary experiments revealed that agitation of *N. dubius* larvae by syringe does affect viability and must be considered in experimental plans. Further preliminary experiments revealed that an infection with 50 *N. dubius* larvae produced the most consistent egg production patterns as measured by techniques used here. Superinfection of groups of mice with either 100 *N. dubius, A. caninum,* or *T. spiralis* indicated that, under the criteria utilized here, only *A. caninum* superinfections adversely affected the initial *N. dubius* worms' ability to persist. Egg production per female worm apparently was enhanced in the group superinfected with *A. caninum.*

No effect on egg viability or development was noted in any of the experimental groups.

LITERATURE CITED

BAKER, N. F. 1954 Trichostrongylodosis. The mouse as an experimental animal. Proc. Book, Am. Vet. Med. As. pp. 185–191.

———— 1955 The pathogenesis of trichostrongyloid parasites; some effects of *Nematospiroides dubius* on the erythrocyte patterns and spleens of mice. Exp. Parasit. **4**: 526–541.

BAYLIS, H. A. 1926 On a trichostrongyloid nematode from the woodmouse (*Apodemus sylvaticus*). Ann. Mag. Nat. Hist. **18**: 455–464.

———— 1927 A further note on *Nematospiroides dubius,* Baylis, 1926 Ann. Mag. Nat. Hist. **20**: 102–105.

COKER, C. M. 1955 Effects of cortisone on *Trichinella* spiralis infections in non-immunized mice. J. Parasit. **41**: 498–504.

COX, H. W. 1952 The effect of concurrent infection with the dog hookworm, *Ancylostoma caninum,* on the natural and acquired resistance of mice to *Trichinella spiralis.* J. Elisha Mitchell Sci. Soc. **68**: 222–235.

EHRENFORD, F. A. 1954a The life cycle of *Nematospiroides dubius* Baylis (Nematoda: Heligmosomidae). J. Parasit. **40**: 480–481.

———— 1954b Effects of dietary protein on the relationship between laboratory mice and the nematode *Nematospiroides dubius.* J. Parasit. **40**: 486.

GOULSON, H. T. 1958 Studies on the influence of prior infection with *Ancylostoma caninum* on the establishment and maintenance of *Trichinella spiralis* in mice. J. Elisha Mitchell Sci. Soc. **74**: 14–23.

KERR, K. B. 1936 Studies on acquired immunity to the dog hookworm, *Ancylostoma caninum.* Am. J. Hyg. **24**: 381–406.

LARSH, J. E., JR., GILCHRIST, H. B., AND GREENBERG, B. G. 1952 A study of the distribution and longevity of adult *Trichinella spiralis* in immunized and non-immunized mice. J. Elisha Mitchell Sci. Soc. **68**: 1–11.

LARSH, J. E., JR., AND KENT, D. E. 1949 The effect of alcohol on the natural and acquired immunity of mice to infection with *Trichinella spiralis.* J. Parasit. 35: 45–53.

LARSH, J. E., JR., AND RACE, G. J. 1954 A histopathologic study of the anterior small intestine of immunized and nonimmunized mice infected with *Trichinella spiralis.* J. Inf. Dis. **94**: 262–272.

MCGEE, G., RUSSOMANNO, R., AND SANDGROUND, J. H. 1957 An expeditious method for counting trichostrongylids and other helminths in the small intestine. J. Parasit. **43**: 315–317.

SPURLOCK, G. M. 1943 Observations on host-parasite relations between laboratory mice and *Nematospiroides dubius,* Baylis. J. Parasit. **29**: 303–311.

STOLL, N. R., AND HAUSHEER, W. C. 1926 Concerning two options in dilution egg counting; small drop and displacement. Am. J. Hyg. **6**: 134–145.

WEINSTEIN, P. P. AND JONES, M. F. 1956 The in vitro cultivation of *Nippostrongylus muris* to the adult stage. J. Parasit. **42**: 215–236.

DESCRIPTION OF *SPLENDIDOFILARIA FLEXIVAGINALIS* N. SP. (NEMATODA: ONCHOCERCIDAE) FROM THE EASTERN CROW, AND KEY TO SPECIES OF THE GENUS *SPLENDIDOFILARIA* SKRJABIN*

JOSEPH JONES, JR.**

Department of Zoology and Entomology, The Ohio State University, Columbus 10

While investigating the parasites of the eastern crow, *Corvus brachyrhynchos brachyrhynchos*, in Ohio, specimens which appear to represent a new species of the genus *Splendidofilaria* were found. This genus now comprises eleven species, including the one described in this paper.

All specimens were fixed in Lavdowsky's AFA fixing reagent, and cleared in glycerin-alcohol. Drawings were made with the aid of camera lucida. Measurements are given in millimeters; the size range of each structure precedes the mean measurement which is placed in parenthesis. The classification used is that of Chabaud and Anderson (1959). The description is based on a study of thirty-five male and fifteen female specimens.

Splendidofilaria Skrjabin, 1923

Generic diagnosis (Chabaud and Anderson, 1959): Anterior extremity without lateral cervical alae. Spicules subequal. Cuticle ornamented or smooth. Pre-esophageal vulva. Cloacal papillae small, arranged in longitudinal rows rather than centered around the cloaca. Parasites of birds.

Splendidofilaria flexivaginalis n. sp.

Description (adults): Cuticle unstriated, without cuticular bosses. Head bearing four submedian papillae and pair of lateral amphids. Esophagus with glandular and muscular portions. Alimentary canal entire, occasionally atrophied near anus.

Male: Body 8.0 to 14.0 (12.0) in length, 0.077 to 0.098 (0.089) in width at level of upper end of intestine. Nerve ring 0.124 to 0.140 (0.136) from anterior end. Excretory pore inconspicuous, 0.175 to 0.189 (0.182) from anterior end. Esophagus 0.660 to 1.05 (0.982) in total length; muscular portion 0.129 to 0.149 (0.137) in length; glandular portion 0.533 to 0.911 (0.743) in length. Cloaca 0.070 to 0.105 (0.088) from caudal end. Tail digitiform, recurved, 0.028 to 0.053 (0.048) in width at cloaca; bearing either four (holotype) or five pairs of linear, symmetrical or asymmetrical, post-cloacal papillae. Spicules subequal. Right spicule 0.085 to 0.126 (0.105) in length. Left spicule 0.067 to 0.095 (0.082) in length.

Female: Body 20.0 to 28.0 (25.0) in length, 0.098 to 0.182 (0.147) in width in region of vulva. Nerve ring 0.119 to 0.180 (0.139) from anterior end. Excretory pore inconspicuous, 0.168 from anterior end. Esophagus 1.15 to 1.70 (1.35) in total length; muscular portion 0.121 to 0.205 (0.144) in length; glandular portion 1.01 to 1.58 (1.24) in length. Anus frequently inconspicuous, 0.098 to 0.145 (0.125) from posterior end. Tail digitiform, tapering posteriorly, 0.053 to 0.075 (0.063) in width at anus. Vulva 0.350 to 0.575 (0.466) from anterior end. The vagina proceeds posteriorly from vulva for distance of 0.060 to 0.210 (0.145), turns anteriorly for distance of 0.259 to 0.450 (0.370), then bends caudally joining bifurcated uterus at 2.6 to 3.5 (3.0) from anterior end. In some specimens, vagina leads straight back to uterus. Uteri parallel; opisthodelphys. Oviducts and ovaries coiled.

Microfilaria (from vagina). Body cylindrical, unsheathed, tapering posteriorly, 0.142 to 0.175 (0.150) in length; 0.003 to 0.006 (0.005) in maximal width. Cuticle with fine transverse striations. Anterior end blunt, free of nuclei; posterior end round, with nuclei. Excretory pore 0.063 to 0.075 (0.068) from anterior end. Excretory vesicle prominent, forming large, clear area 0.043 to 0.062 (0.053) from posterior end. Excretory cell inconspicuous, immediately behind vesicle. Genital cell 0.034 to 0.043 (0.037) from posterior end.

Host: Corvus brachyrhynchos brachyrhynchos, Eastern Crow

Site of infection: Enclosed in mesentery surrounding posterior vena cava, near spleen.

Type locality: Montgomery County, Ohio.

Holotype (male) U. S. N. M. Helm. Coll. No. 39047.

Allotype (female) U. S. N. M. Helm. Coll. No. 39047.

Paratypes (five males and three females) U. S. N. M. Helm. Coll. No. 39048 and Ohio State Univ. Helm. Coll. No. P108.

DISCUSSION

Splendidofilaria flexivaginalis n. sp. most closely resembles *Splendidofilaria columbigalli-*

Received for publication August 17, 1960.

* Taken from a dissertation submitted to the Graduate School of The Ohio State University in partial fulfillment of the requirements for the degree Doctor of Philosophy.

** Present address: Saint Augustine's College, Raleigh, N. C.

nae (Augustine, 1937). It differs from the latter species in the arrangement and sometimes in the number of caudal papillae; in the shape of the vagina which may be curved towards the anterior end, forming a vaginal loop; and in the size of the spicules and other organs. Moreover, the microfilaria of *S. columbigallinae* is sheathed, while that of *Splendidofilaria flexivaginalis* is not sheathed.

Variations in the number and arrangement of caudal papillae were frequently observed in various specimens of *S. flexivaginalis*. In an examination of 35 male specimens, I found 10 which possessed 5 pairs of caudal papillae while the remainder had 4 pairs.

Specimens of *S. flexivaginalis* were found in 33 percent of the 339 crows examined during the course of this investigation; the number of parasites found in each bird varied from 1 to 18. Immature crows were more frequently infected with the adult stage of this parasite than were mature crows. A greater percentage of the crows harbored this filarial worm during the summer months than in the winter.

Anderson and Chabaud (1959) recognized 10 valid species of the genus *Splendidofilaria;* these and *S. flexivaginalis* n. sp. are presented in the following key.

Key to the Species of Genus
Splendidofilaria *Skrjabin, 1923*
(*Based on original descriptions*)

1(7) Cuticular bosses present 2

2(4) Male with pre- and post-cloacal papillae. Esophagus divided into muscular and glandular portions 3

3(2) Male with two pairs of pre-cloacal, two pairs of ad-cloacal and four pairs of post-cloacal papillae. Body length: male 23.0; female 34.0. Length of spicules 0.063 and 0.071. Opisthodelphys ..... *S. gedoelsti* Travassos, 1926

4(2) Posterior end of male bearing only post-cloacal papillae. Esophagus not divided into muscular and glandular portions 5

5(6) Male with four pairs of caudal papillae. Body length: male 7.4; female 18.9. Length of spicules 0.068 and 0.077. *S. pawlowskyi* Skrjabin, 1923

6(5) Male with two pairs of caudal papillae. Body length: male 14.0; female 29.0. Length of spicules 0.045 and 0.040. *S. verrucosa* Oschmarin, 1950

7(1) Cuticular bosses absent 8

8(17) Esophagus divided into muscular and glandular portions 9

9(12) Male with pre- and post-cloacal papillae 10

10(11) Male with one pair of pre-cloacal and seven pairs of post-cloacal papillae. Body length: male 9.0; female 21.0. Length of spicules 0.062 and 0.071. Amphidelphys. *S. periarterialis* (Caballero, 1948) Chabaud and Choquet, 1953

11(10) Male with four pairs of pre-cloacal and three pairs of post-cloacal papillae. Body length: male 27.0 to 33.0; female 65.0 to 83.0. Length of spicules 0.073 to 0.084 and 0.091 to 0.094. Opisthodelphys. *S. braziliensis* (Yeh, 1957) Anderson and Chabaud, 1959

12(9) Posterior end of male bearing only post-cloacal papillae 13

13(14;15;16) Male with four pairs of caudal papillae. Body length: male 13.0; female 20.0 to 23.0. Length of spicules 0.070 to 0.080. Opisthodelphys. Microfilaria sheathed. *S. columbigallinae* (Augustine, 1937) Chabaud and Choquet, 1953

14(13;15;16) Male with either four or five pairs of caudal papillae. Body length: male 8.0 to 14.0; female 20.0 to 28.0. Length of spicules 0.085 to 0.126 and 0.067 to 0.095. Opisthodelphys. Vagina straight or curved. Microfilaria unsheathed. ..... *S. flexivaginalis* n. sp.

15(13;14;16) Male with three pairs of caudal papillae. Body length: male 18.0 to 20.0; female 27.0 to 31.5. Spicules with denticulate markings, 0.069 to 0.071 and 0.060 to 0.062 in length. Opisthodelphys. *S. singhi* (Ali, 1956) Anderson and Chabaud, 1959

16(13;14;15) Male with three pairs of caudal papillae. Body length: male 15.0 to 18.0; female 23.0 to 25.0. Length of spicules 0.050 to 0.060 and 0.070 to 0.080. Opisthodelphys. *S. sinensis* (Li, 1933) Chabaud and Choquet, 1953

17(8) Esophagus not divided into muscular and glandular portions. Posterior end of male bearing only post-cloacal papillae 18

18(19) Male with three pairs of caudal papillae. Body length: male 9.0 to 11.0; female 28.0. Length of spicules 0.070 to 0.080. Amphidelphys. *S. bosei* (Chandler, 1924) Chabaud and Choquet, 1953

19(18) Male with two pairs of caudal papillae. Body length: male 6.1; females unknown. Length of spicules 0.062 and 0.078. *S. gretillati* Chabaud, Anderson and Brygoo, 1959.

ACKNOWLEDGMENTS

The writer wishes to acknowledge the constructive criticisms of Dr. Joseph N. Miller under whose supervision this investigation was undertaken. Appreciation is expressed to Dr. John L. Crites for the helpful suggestions which he gave during the preparation of this paper.

LITERATURE CITED

ANDERSON, R. C. AND CHABAUD, A. G. 1959 Remarques sur la classification des Splendidofilariinae. Ann. Parasit. **34**: 53–63.

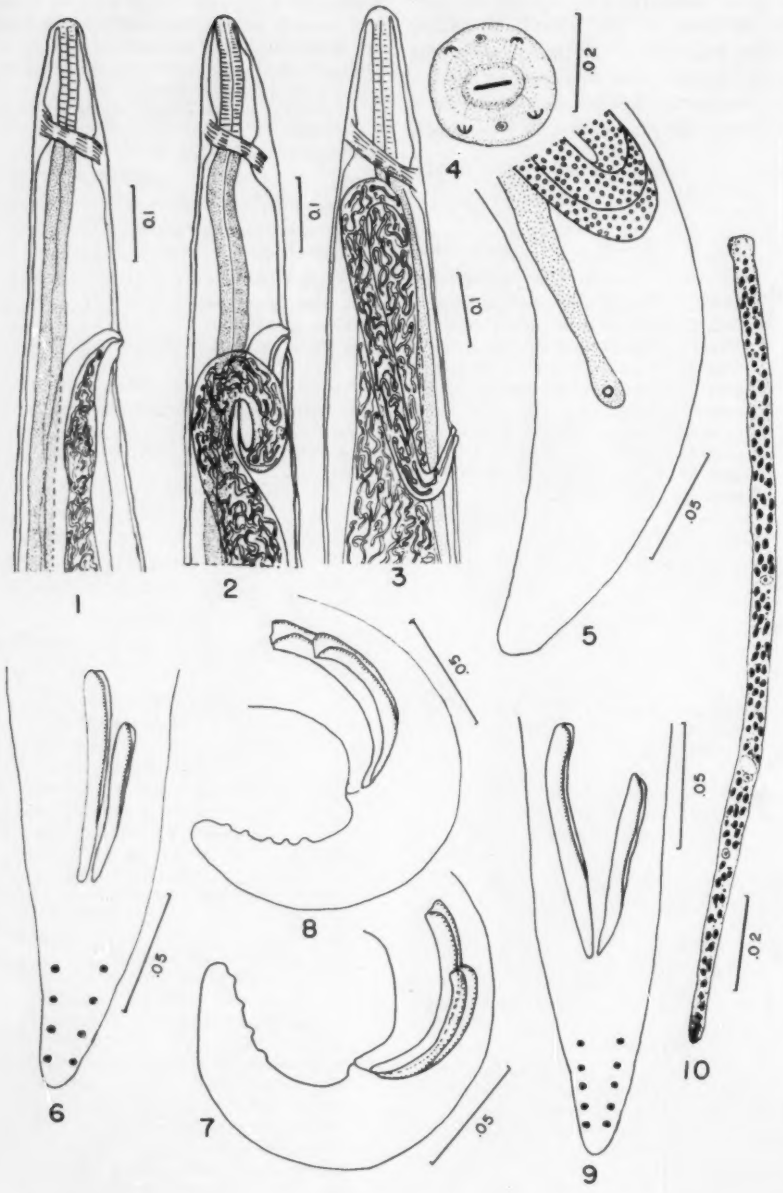
CHABAUD, A. G. AND ANDERSON, R. C. 1959 Nouvel essai de classification des filaires (Superfamille des Filarioidea) II. Ann. Parasit. **34**: 64–87.

EXPLANATION OF PLATE I

Splendidofilaria flexivaginalis n. sp.

FIGURE 1. Female with straight vagina, lateral view (paratype).
FIGURE 2. Female with curved vagina, lateral view (paratype).
FIGURE 3. Female with vaginal curve extending above vulva, lateral view (allotype).
FIGURE 4. Head of female, en face view.
FIGURE 5. Caudal end of female, ventral view (paratype).
FIGURE 6. Caudal end of male with four pairs of papillae, ventral view (holotype).
FIGURE 7. Same as figure 6, lateral view (holotype).
FIGURE 8. Caudal end of male with five pairs of papillae, lateral view (paratype).
FIGURE 9. Same as figure 8, ventral view (paratype).
FIGURE 10. Microfilaria.

PLATE I



INCIDENCE OF MICROFILARIAE IN SOME OHIO BIRDS AND DATA ON THE HABITS OF A POSSIBLE VECTOR*

EDWIN J. ROBINSON, JR.
Department of Biology, Kenyon College, Gambier, Ohio

Information on the extent of filarial worm infections in wild populations is sketchy, and the life history of only one avian filarial parasite is known (Anderson, 1956). There have been numerous surveys of avian blood parasites, of which three may be mentioned. Love, Wilkin and Goodwin (1953) found 4 percent microfilarial infection rate in 1,068 birds examined, 8 percent of 87 blue jays being infected; Saunders (1955) found microfilariae in 24 species and subspecies of birds, but gave no infection rates; and Bennett and Fallis (1960) report a 3.4 percent infection rate in 3,004 birds.

These surveys probably do not discover many of the infections actually present, since the avian microfilariae studied in this respect usually are nocturnally periodic, as the writer (Robinson, 1954a; 1955) and others (e.g., Odetoyinbo and Ulmer, 1960) have found. The juvenile worms live in the lungs, where they are readily found. They are absent from peripheral and heart blood in the daytime. Since this is the usual source of blood for examination, only stray or incompletely periodic microfilariae are discovered. For example, the writer collected in the same area as Love et al (op. cit.), but by examination of lung smears found a general infection rate of 12 percent, and a 65 percent rate in blue jays (Robinson, 1954a; 1954b).

Accurate knowledge of the natural distribution of filarial worms among birds is useful in studies on filarial life histories, since epizootiological information can suggest what kind of vectors to seek, as was clearly stated by Bennett and Fallis (op. cit.). That was in fact the reason for the survey reported here, since efforts are being made to confirm the writer's hypothesis that avian filarial infections are transmitted from parent to nestling through the bites of *Culicoides* gnats (Robinson, 1955).

A similar but more extensive survey of birds in Georgia has been reported (Robinson, 1954a; 1954b). This appears to be the only accurate (i.e., lung examination) record of microfilarial infections in a sizeable sampling of numerous species. The present survey covers 514 birds of 60 species, nearly all of which nest in central Ohio, and some of which are year-'round residents. Thirty-eight of the species also were collected in Georgia, offering an opportunity for comparison of infection rates.

MATERIALS AND METHODS

The method of collection and examination of the birds, and the preparation of the microfilariae for study, was identical to that described earlier (Robinson, 1954a). The collections were made with some regularity during June, July, and part of August 1959 and 1960, mostly in three permanent study areas. Each is over 500 acres in extent and includes ponds, streams, seepages, hardwood groves, underbrush, barns and outbuildings, and tilled and untilled fields. Some birds were collected at random from likely spots within 5 miles of Gambier and the three permanent areas. Most of the bird habitats in central Ohio are represented. Rare, large, and game birds were largely ignored, so the 60 species include only some of the kinds of birds nesting in the vicinity.

Insects were light-trapped nightly in the three main study areas during June and July 1960. The traps were a standard type, and were hung in bushes or trees at heights varying from 2 to 8 feet.

RESULTS

Table 1 gives a digest of the results of the examinations for microfilariae. A limited number of heavily infected birds was minutely examined for adult worms, but none were found.

DISCUSSION

The findings in the Georgia and Ohio surveys may be compared in several ways. The most outstanding difference between the two is in the blue jay infection rates. Two hundred and sixty-nine of 497 Georgia jays carried filarial worms, but only 2 of 47 in the present

Received for publication October 27, 1960.

* This work was supported by grant E-2583 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

collection. The microfilariae in these were morphologically identical, upon routine examination, to those usually found in the Georgia blue jays.

The reason for the relative freedom of Ohio jays from these parasites is not apparent. The existence of some infected birds (one each summer) proves the worm is locally present. Whether the infected birds were recent migrants into the area cannot be determined, but certain conditions indicate that in each case the birds were immature adults living very close to the nesting sites which produced them. As the vector is unknown, scarcity of the intermediate host cannot be demonstrated. The writer views *Culicoides* as a likely vector, and this gnat is one of the most common insects of any kind entering the light traps. Other species of birds become infected, so suitable filarial worm vectors are present. Therefore, either *Culicoides* is not a vector, or the blue jay filarial worm can maintain itself even where its population density is remarkably low. In the latter case, high transmission efficiency is indicated.

There are too few crows in the present collection to compare infection rates. Since three of four were infected, and infections were found in both years, the local crow population probably is as heavily infected as the Georgia crows. The microfilariae appear to be identical with those in the Georgia birds.

TABLE I. *Microfilarial infections in lungs of Ohio birds.*

Species*	Common name	No. examined	No. infected
Accipitridae			
Accipiter cooperii	Cooper's hawk	1	0
Apodidae			
Chaetura pelagica	Chimney swift	1	0
Ardeidae			
Butorides virescens	Eastern green heron	2	0
Cathartidae			
Cathartes aura septentrionalis	Turkey vulture	1	1
Corvidae			
Corvus brachyrhynchos	Common crow	4	3
Cyanocitta cristata	Blue jay	47	2
Cuculidae			
Coccyzus americanus	Yellow-billed cuckoo	6	2
Fringillidae			
Pipilo erythrophthalmus	Towhee	10	0
Spizella pusilla	Field sparrow	13	0
Melospiza lincolnii	Lincoln's sparrow	1	0
M. melodia	Song sparrow	34	2
Passerella iliaca	Fox sparrow	4	0
Poaecetes gramineus	Vesper sparrow	1	0
Junco hyemalis	Slate-colored junco	1	0
Zonotrichia albicollis	White-throated sparrow	9	1
Hirundinidae			
Hirundo rustica	Barn swallow	2	0
Icteridae			
Sturnella magna	Eastern meadowlark	2	0
Agelaius phoeniceus	Red-winged blackbird	31	2
Dolichonyx oryzivorus	Bobolink	3	0
Icterus spurius	Orchard oriole	2	0
I. galbula	Baltimore oriole	7	0
Quiscalus quiscula	Purple grackle	2	1**
Molothrus ater	Brown-headed cowbird	1	0
Mimidae			
Dumetella carolinensis	Catbird	24	7 **
Paridae			
Baeolophus bicolor	Tufted titmouse	25	0
Parus carolinensis	Carolina chickadee	11	0
Paridae			
Geothlypis trichas	Maryland yellowthroat	2	0
Icteria virens	Yellow-breasted chat	3	1
Vermivora pinus	Blue-winged warbler	1	0
V. ruficapilla	Nashville warbler	1	0
Picidae			
Melanerpes carolinus	Red-bellied woodpecker	6	0
M. erythrocephalus	Red-headed woodpecker	1	0
Dendrocopos pubescens	Downy woodpecker	20	0
D. villosus	Hairy woodpecker	1	0
Colaptes auratus	Yellow-shafted flicker	6	1**
Ploceidae			
Passer domesticus	English sparrow	57	0
Spinus tristis	Eastern goldfinch	12	1
Hesperiphona vespertina	Evening grosbeak	1	0
Phasianidae			
Colinus virginianus	Bobwhite	1	0
Sittidae			
Sitta carolinensis	White-breasted nuthatch	17	0
Sturnidae			
Sturnus vulgaris	Starling	9	1
Sylviidae			
Polioptila caerulea	Blue-gray gnatcatcher	3	0
Thraupidae			
Piranga olivacea	Scarlet tanager	2	1
P. rubra	Summer tanager	2	0
Passerina cyanea	Indigo bunting	12	1
Pyrrhuloxia cardinalis	Cardinal	19	3**
Tyrannidae			
Tyrannus tyrannus	Eastern kingbird	4	1
Myiarchus crinitus	Crested flycatcher	7	4**
Empidonax minimus	Least flycatcher	1	0
Contopus virens	Eastern wood pewee	29	4**
Sayornis phoebe	Eastern phoebe	4	0
Tytonidae			
Tyto alba	Barn owl	1	0
Troglodytidae			
Thryothorus ludovicianus	Carolina wren	2	0
Cistothorus platensis	Short-billed marsh wren	4	0
Troglodytes aëdon	House wren	1	0
Turdidae			
Turdus migratorius	American robin	11	1
Sialia sialis	Eastern bluebird	4	0
Hylocichla ustulata	Olive-backed thrush	2	0
Vireonidae			
Vireo olivaceus	Red-eyed vireo	8	1
V. flavifrons	Yellow-throated vireo	6	0
Unidentified birds		9	0
	Totals	514	41
	Percent infected ..	8	

* Named according to Blair, Blair, Brodkorb, Cagle and Moore "Vertebrates of the United States," McGraw-Hill, 1957.

** Species in which at least one infected juvenile was found.

Infection rates in the following species and families are not greatly different in the two regions: cuckoo, red-winged blackbird, tufted titmouse, chickadee, downy woodpecker, English sparrow, cardinal, red-eyed vireo; Fringillidae (except for the towhee), Icteridae, Mimidae, Paridae, Picidae, Thraupidae, and Tyrannidae. Large differences are seen in the blue jay, towhee, crested flycatcher; Turdidae and Vireonidae.

If the species collected in exceptional numbers are excluded from calculation of the general infection rates (blue jays and crows in Georgia, blue jays and English sparrows in Ohio), then over 12 percent of the Georgia and 9 percent of the Ohio birds were infected. While this is not a substantial difference, it could be a reflection of more favorable transmission conditions in the warmer climate.

Fifty-six of the 57 English sparrows were collected in the first season. Their inclusion would make comparison of the two seasons impossible. If they are excluded, the total infection rates for the two collecting seasons in the present survey are 8 percent and 10 percent for 227 and 230 birds, respectively.

The much more extensive survey of Bennett and Fallis (op. cit.) showed only 3.4 percent of 3,004 birds to have microfilariae, when stained films or fresh peripheral blood were examined. As they recognize, the actual total infection rate must be higher, because of the nocturnal periodicity of the worms. It is reasonable to believe that the real rate in the 94 species of birds in their survey must have been at least in the range of those reported in the surveys by this writer. The remarkable consistency in general infection rates, year by year, or area by area, among well over 100 avian species shows that the transmission mechanism is both stable and efficient.

Discovery of patent infections in juvenile birds of six species corresponds quite well with a similar finding in 11 species in the Georgia survey. Only one of the six (cardinal) is included in the earlier list, so at least 16 species of birds collected by the writer may be and sometimes are infected in the nestling stage. Bennett and Fallis (op. cit.) found microfilariae in a number of juvenile hosts, including four species to be added to the 16 in the writer's records. These are: myrtle warbler, Blackburnian warbler, olive-backed thrush, and American robin.

Two of the juvenile infections are of particular interest, because of the certainty that the birds were very young, even though their exact age in days cannot be stated. The flicker was shot near the nest hole, in the company of other flickers. It was not fully feathered, and it may be concluded that it contracted the infection as a nestling, and that the filarial species involved matures very rapidly.

Two of the infected flycatchers were part of a family group, which indicates that the juveniles were only a few weeks old. Two juveniles and one adult were shot, and the adult and one juvenile were infected. The young bird must have been infected before it left the nest, and the adult could have been the source of the parasite.

The rate of infection is partly a function of the number and activity of the vectors. The consistency of general infection rates implies that the vectors are common, always present during the transmission season, and regularly active in the habitats of the avian hosts. The consistently low or high rates in certain groups (as perhaps in the fringillids or mimids) could be due to specific ecological conditions determining the frequency with which the vector invades the bird breeding areas and nests. This would involve a combination of bird nesting habits, especially with reference to height, and the vector flying and biting habits.

Bennett (1960) trapped different species of *Culicoides* at different heights when he used live birds for bait. He also distinguished some feeding preferences in the flies, with *Culicoides sphagnumensis* and *C. stilobezzioides* preferring woodland birds. Bennett and Fallis (op. cit.) concluded that transmission of the avian blood parasites was during the breeding season, by biting flies, and resulted in the infection of young birds. The writer came to a similar conclusion for filarial infections as a result of the earlier survey, and an epizootiological study of corvid filarial worms (Robinson, 1955). Each bird species habitually nests at a typical height, and this collector came to expect more infected specimens from among the woodland birds than from nest-hole and open-field breeders, and the high arboreal species.

In the present study, all the light traps caught *Culicoides,* but its apparent distribution was uneven. It was common in shrubby woods

inhabited by catbirds with a fairly high rate of infection, but was virtually absent over grassy plots or fields immediately adjacent. The height distribution was not determined in this preliminary work. With one exception, the gnat was more common near possible breeding places in seepages or rotting organic material than near running water or ponds. The flying, biting, and breeding habits of this possible vector will be more extensively studied during the next breeding season.

The facts just related and deductions from them are consistent with the hypothesis of transmission of avian filarial worms which the writer has proposed, although definitive proof must be found in life history studies and increased knowledge of the vector's habits.

SUMMARY AND CONCLUSIONS

Eight percent of 514 birds of 60 species collected in central Ohio were infected with microfilariae. This record is compared with a similar but larger collection made in Georgia. With a few exceptions, which include the blue jay, there is a general coincidence in infection rates among species and families common to the two areas.

The possibility of a relationship between the bird habitat and the rate of infection was discussed, and some preliminary findings about the distribution of a possible vector, *Culicoides*, were presented.

It is concluded that transmission rates for filarial worms are consistent enough in different geographical areas and in different years to indicate that the vector is wide-spread, common and active; and that the habits of the vector and the nesting habits of the birds are major determining factors in transmission of the worms. Support was found for the hypothesis that filarial infections in birds are familial, and contracted by nestlings.

LITERATURE CITED

ANDERSON, R. C. 1956 The life cycle and seasonal transmission of *Ornithofilaria fallisensis* Anderson, a parasite of domestic and wild ducks. Can. J. Zool. **34**: 485–525.

BENNETT, G. F. 1960 Some ornithophilic blood-sucking Diptera in Algonquin Park, Ontario, Canada. Can. J. Zool. **38**: 377–389.

BENNETT, G. F. AND FALLIS, A. M. 1960 Blood parasites of birds in Algonquin Park, Canada, and a discussion of their transmission. Can. J. Zool. **38**: 261–273.

LOVE, G. J., WILKIN, S. A. AND GOODWIN, M. H., JR. 1953 Incidence of blood parasites in birds collected in southwestern Georgia. J. Parasit. **39**: 52–57.

ODETOYINBO, J. A. AND ULMER, M. J. 1960 Microfilarial periodicity of *Splendidofilaria quiscali* (von Linstow, 1904) n. comb. (Nematoda: Onchocercidae). J. Parasit. **46** (supp.): 18–19.

ROBINSON, E. J., JR. 1954a Notes on the occurrence and biology of filarial nematodes in southwestern Georgia. J. Parasit. **40**: 138–147.

———— 1954b Additional data on filarial worm infections in vertebrates of southwestern Georgia. J. Parasit. **40**: 690.

———— 1955 Observations on the epizootiology of filarial infections in two species of the avian family Corvidae. J. Parasit. **41**: 209–214.

SAUNDERS, D. C. 1955 The classification of microfilariae in birds. *Avifilaria tyrannidarum* and *A. fringillidarum*, two new species. Tr. Am. Micr. Soc. **74**: 37–45.

EXTENSIVE ABNORMALITIES IN LARVAL TREMATODE INFECTION*

KATHLEEN L. HUSSEY AND WALTER B. STAHL

Abnormal larval stages of trematodes in the form of partial twinning of the organism have occasionally been seen. Hoffman and Janer (1936) and Janer (1941) reported such abnormalities in two and one specimens, respectively, of miracidia of *Schistosoma mansoni*. Najarian (1954) noted a single partially twinned daughter redia of *Echinoparyphium flexum*. Schell (1960) described a twinned redia of *Notocotylus quinqueserialis*, and the present authors (unpublished data) found 3 partially twinned rediae in an echinostome infection in a *Physa parkeri* collected from Douglas Lake, Michigan. Pelseneer (1906) figured a specimen of *Cercaria setifera* with trifid tail, while a partially twinned cercaria was reported by Mathias (1930) in an unidentified holostome infection from *Bithynia tentaculata* and by Hussey (1941) in *Alloglossidium corti*. In addition to minor abnormalities in cercariae, Kuntz (1948) described almost complete twinning in a cercaria of *Notocotylus urbanensis* and various degrees of twinning in three cercariae of *Schistosoma mansoni*. In all instances cited, one or only a very few of the larval stages in a given trematode infection showed the abnormality.

During the course of a study of larval trematodes, one specimen of *Stagnicola emarginata canadensis* from a roadside ditch at Alanson, Michigan, harbored an unidentified stylet infection with an unusually large number of abnormal embryos in the daughter sporocysts. It was apparent that these abnormal embryos were widely distributed throughout the whole trematode infection. Twenty daughter sporocysts picked at random and removed entire, then opened and their contents noted, had from 13 to 88 percent of abnormal embryos.

The majority of the abnormalities appeared as varying degrees of duplication of the posterior regions of developing cercariae (fig. 1, 1–4) although many cercariae also had extraneous appendages or projections (6). One specimen (5) had a duplication of both anterior and posterior ends of the body while some showed a tripling of the posterior end (7). In addition to these cercarial embryos, the daughter sporocysts also contained many elongate embryos (8–11) some of which appeared to have a cavity containing cellular masses (8) suggesting the structure of young daughter sporocysts. A few of these showed some evidence of partial twinning (10) or had extraneous appendages or projections.

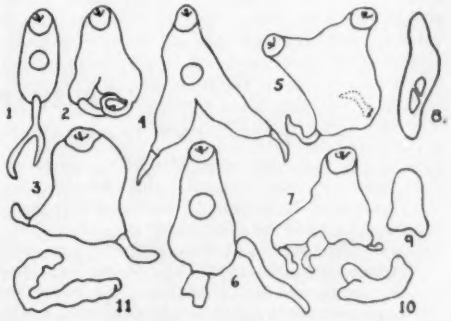


FIGURE 1. Free-hand outline drawings from living material and photographs.

The snail carrying this infection had been isolated and kept in the laboratory for 24 days during which time normal stylet cercariae emerged.

Whether the cause of such abnormalities is genetic or a result of injury or physiological changes in the host at critical points of development of the trematode, as suggested by Kuntz (1948), is still a matter for conjecture. In the present instance we may postulate that some of

* Contribution from the University of Michigan Biological Station and the School of Public Health and Administrative Medicine of Columbia University. This investigation was supported (in part) by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

the germinal cells in the developing daughter sporocysts within the mother sporocyst were affected, perhaps those germinal cells within the germinal mass that had not started to develop into embryos. These daughter sporocysts, after their migration into the snail's tissues, then produced both abnormal embryos and some apparently completely normal cercariae which emerged from the snail host. Although these abnormal cercariae were alive and showed a certain amount of movement, they would probably never have emerged naturally from the snail.

REFERENCES

HOFFMAN, W. A. AND JANER, J. L. 1936 Miracidial twinning in *Schistosoma mansoni* (Trematoda: Schistosomatidae). Proc. Helm. Soc. Wash. **3**: 62.

HUSSEY, K. L. 1941 Partial twinning in a stylet cercaria. Parasit. **27**: 92–93.

JANER, J. L. 1941 Miracidial twinning in *S. mansoni*. J. Parasit. **27**: 93.

KUNTZ, R. E. 1948 Abnormalities in development of helminth parasites with a description of several anomalies in cercariae of digenetic trematodes. Proc. Helm. Soc. Wash. **15**: 73–77.

MATHIAS, P. 1930 Sur une cercaire monstre double du type lambdoïde. Ann. Parasit. **8**: 147–150.

NAJARIAN, H. H. 1954 Developmental stages in the life cycle of *Echinoparyphium flexum* (Linton, 1892) Dietz, 1910 (Trematoda: Echinostomatidae). J. Morph. **94**: 165–197.

PELSENEER, P. 1906 Trématodes parasites de mollusques marins. Bull. Sci. France et Belg. **40**: 165.

SCHELL, S. C. 1960 A case of conjoined twin rediae. J. Parasit. **46**: 448.

RESEARCH NOTE

COOPERIA PUNCTATA IN AXIS DEER (*CERVUS AXIS*) IN HAWAII.

In the course of a helminth survey of vertebrates in the Hawaiian Islands during October 1960 the author examined three mature axis deer (*Cervus axis*). In one deer each from the Islands of Lanai and Molokai a few typical mature specimens of *Cooperia punctata* were recovered from the small intestine.

Most species of *Cooperia* have been recorded from cattle, sheep, goats, antelope, and the horse. Dikmans (1934, Proc. Helm. Soc. Wash. **1**: 63) recorded *Cooperia* sp. from *Odocoileus virginianus* in New York State and more recently, *O. hemionus columbianus* has been reported as a host for *C. mcmasteri* in California (Allen et al, 1958, J. Parasit. **44**: 503–506).

C. punctata is a common parasite of calves in Hawaii (Alicata, 1960, Am. J. Vet. Res. **21**: 410–415). It is likely that the deer acquired the infection from foraging in localities formerly grazed by cattle. In one area where the parasites were found in the deer, cattle had been removed at least 5 years previously. Heavy infections with *Cooperia* in calves is known to produce scouring, debilitation, and even death. To what extent young deer may be affected remains to be determined. The above observation leads to believe that under field conditions deer can serve as a reservoir host of cooperid infection.

This study was supported by research grant E-3072 from the National Institutes of Health, U. S. Public Health Service. The cooperation of the Hawaii Department of Agriculture and Conservation is gratefully acknowledged.—LAWRENCE R. ASH, *Department of Parasitology, University of Hawaii, Honolulu 14.*

AUSTROBILHARZIA PENNERI, A NEW SCHISTOSOME FROM MARINE SNAILS*

ROBERT B. SHORT AND RHODES B. HOLLIMAN**
Department of Biological Sciences, Florida State University, Tallahassee

During the summer of 1959 a dermatitis, suspected of being schistosome induced, was contracted by Mr. Horace G. Loftin while wading in shallow water at Shell Point, Franklin Co., Florida, on the Northern Gulf coast. Examination by one of us (R. B. H.) of a large number of the predominant snail, *Cerithidea scalariformis*, from the area revealed a schistosome cercaria. The larva has been described elsewhere, and its ability to produce dermatitis has been confirmed experimentally (Holliman, in press). Parakeets, chicks, and pigeons exposed to cercariae yielded adult worms belonging to the genus *Austrobilharzia* Johnston, 1917. A muscovy and a mallard duck, also exposed, were negative at autopsy. The adult worms are here described as *Austrobilharzia penneri* n. sp. in honor of Dr. Lawrence R. Penner.

Specimens were placed in 0.85 percent saline on removal from hosts and were then fixed in A.F.A., some with, others without, coverglass pressure. Whole mounts were stained with Ehrlich's acid haematoxylin or Semichon's acetocarmine; sections of a few specimens were stained with iron haematoxylin and eosin.

The following description is based chiefly on whole mounts of 70 males and 18 females from parakeets, chicks, and pigeons. Certain morphological details were confirmed in serial sections. All drawings were made with the aid of camera lucida. Measurements are in millimeters, unless stated otherwise. Averages precede ranges in parentheses.

The authors wish to express their appreciation to Mr. Horace G. Loftin for his aid in reporting the dermatitis to them and collecting snails. We also thank Mr. Loftin and Mr. Edwin Tyson for recovering and preparing for study adult worms from pigeons, and Dr. Allen McIntosh for lending us type material of *Microbilharzia chapini*.

DESCRIPTION

Male: Measurements are of whole mounts from two groups of sexually mature specimens recovered from chicks: 18 worms 15 days of age fixed under slight coverglass pressure; 12 worms 31 days of age fixed without pressure. Sizes and proportions of the specimens in the two groups are very similar, e.g., the length of specimens 15 days of age is 2.032 (1.487 to 2.935) as compared with 2.082 (1.340 to 2.613) for those 31 days of age. Other measurements are from worms of both ages, unless stated otherwise.

Acetabulum about 0.183 (0.126 to 0.224; 20 worms) in diameter when spread, usually situated one-sixth to one-fifth body length from the anterior tip, but that distance can be one-eighth to one-fourth body length. Lining of acetabulum, oral sucker and gynecophoric canal with fine spines which also occur on edge of body fold opposite genital pore (fig. 6). Spines in dorsal part of canal limited to transverse ridges (fig. 7), which disappear toward edges of canal with the spines becoming more evenly distributed; cuticle otherwise devoid of spines. Oral sucker diameter 0.158 (0.120 to 0.177; 20 worms). Esophageal glands at posterior end of esophagus. Gut bifurcating immediately anterior to acetabulum with ceca uniting posterior to testes, usually at about one-third body length from posterior end. Intestine posterior to testes with various patterns of anastomosis (figs. 11–13), ending in common cecum before reaching posterior tip of body. Short gut diverticula, especially prominent posterior to testes.

Testes 11 to 16 in 51 worms; 37 with 12–14 testes each; their shape variable (figs. 1, 10). Testes arranged in an irregular double row, usually occupying a zone about one-third the body length, lying mostly in middle body third; extending a short distance into the anterior third in 21 of 30 specimens, into the posterior third in four others, into anterior and posterior thirds in one, and confined to middle third in four. Short sperm ducts uniting testes, a single duct passing anteriorly to join ovoid seminal vesicle about midway between anterior-most testis and posterior border of acetabulum. Ejaculatory duct leading from vesicle laterally and ventrally within cirrus pouch which contains prostate cells and a cirrus with fine

Received for publication September 6, 1960.

* This investigation was supported in part by research grant E-1142 from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, Public Health Service.

** Present address: Department of Biology, Jacksonville University, Jacksonville, Florida.

spines on its distal end when everted (figs. 2, 3). Cirrus pouch usually more elongate than in flattened specimen shown in figure 2. Genital pore on inner surface of right or left gynecophoric fold near margin where fold projects as a flattened lobe devoid of spines, in contrast to opposite fold (fig. 6).

Female: Unless stated otherwise, measurements are from worms 15 to 31 days of age reared in chicks and fixed on slides without pressure. Unflattened females, 12 and 14 days of age from a parakeet have very similar proportions to those from chicks, but are of smaller average size.

Body length of 10 worms 2.381 (1.876 to 3.062); maximum width of 8 specimens averaging 0.200, with body tapering gradually toward anterior end from ovarian level, bluntly pointed posteriorly. Expanded acetabulum about 0.066 in diameter; situated about one-twentieth to one-seventh body length from anterior end, depending chiefly on contraction of the preacetabular region. Cuticular spines restricted to lining of suckers. Oral sucker about 0.061 in diameter; usual gland cells at posterior end of esophagus. Gut bifurcating immediately anterior to acetabulum; ceca uniting immediately posterior to ovary to form common cecum which is more voluminous than in the male and extending posteriorly without anastomosis between vitelline follicles almost to posterior end of body. Excretory vesicle small, V-shaped.

Ovary elongate, spirally coiled in a zone 0.301 (0.228 to 0.369; 11 specimens) long, wider posteriorly where the ovary usually occupies one-half or more of the body width. Location of ovary in 17 worms (7 from a parakeet): within anterior third of body in 10 specimens; extending into the middle third, usually only slightly, in 7 specimens; posterior margin 42 percent of body length from anterior end in one specimen with attenuated anterior end. Oviduct leaves posterior end of ovary (fig. 5) as a convoluted tube extending anteriorly to join ootype near acetabulum. Uterus short; genital pore midventral, immediately posterior to acetabulum. Ovoid to spherical seminal receptacle near posterior end of ovary; Laurer's canal joins oviduct a short distance anterior to receptacle and opens dorsal to posterior region of ovary.

Vitellaria occupy most of body posterior to ovary and lateral to cecum, with duct extending anteriorly, ventrally, and ventrolaterally to ovary, joining oviduct at ootype. Mehlis' gland not prominent, its ducts entering posterior portion of ootype.

Two specimens ovigerous, with a single egg in the ootype. Living eggs in chick gut tissues and scrapings usually broadly ovoid (fig. 8), with terminal spine or knob about 10 microns long and varying in shape (fig. 9), usually being thinner and more pointed on recently deposited eggs than on those containing miracidia. Twenty recently deposited eggs containing evident vitelline cells measured (excluding spine) 69.8×60.9 microns (63.2 to 79.0×58.5 to 66.4), 20 with apparently mature miracidia 102.4×90.1 microns (88.5 to 113.8×79.0 to 101.1).

Hosts: Intermediate, *Cerithidea scalariformis* Say; natural definitive, unknown; experimental definitive, chick (*Gallus gallus* (Linnaeus)) parakeet (*Melopsittacus undulatus* Shaw), and pigeon (*Columba livia* Gmelin).

Locality: Gulf coast, Franklin Co., Florida.

Type specimens: Deposited in U.S.N.M. Helm. Coll.: Holotype, (male) No. 39070, allotype (female) No. 39070; paratypes in author's collections.

DISCUSSION

There is some uncertainty concerning the species assigned to the genus *Austrobilharzia* Johnston, 1917, partly because of inadequate descriptions. Price (1929) listed only *Austrobilharzia terrigalensis* Johnston, 1917, and erected a new genus, *Microbilharzia*, for *M. chapini* which apparently differs from *A. terrigalensis* chiefly in the gynecophoric canal's extent anterior to the acetabulum and the males being shorter than the females. Price's statement that the uterus of *M. chapini* is long is probably an error. In our examination of type material neither the ootype nor the uterus was seen, but the main vitelline duct was traced well forward, indicating that the uterus is short. Stunkard and Hinchliffe (1952) determined the adult of *Cercaria variglandis* Miller and Northrup, 1926, and concluded that it is conspecific with *M. chapini*, and that the name should be *Microbilharzia variglandis* (Miller and Northup, 1926). Penner (1953), after comparing adults of *C. variglandis* from experimental hosts and from naturally infected birds with type material of *M. chapini*, suppressed the genus *Microbilharzia* as a synonym of *Austrobilharzia*. Baerup's (1956) redescription of adult *A. terrigalensis* supports Penner's opinion. The name *Austrobilharzia variglandis* (Miller and Northup, 1926) Penner, 1953, is considered by the present authors to be correct for the adult of *C. variglandis*.

Chu and Cutress (1954) identified as *Austrobilharzia variglandis* a schistosome species in Hawaii, but found it difficult to reconcile differences between ceca of *M. chapini* and *A. variglandis* from Hawaii and the United States, and suggested that such differences are of specific value. If this is correct, *M. chapini* Price, 1929, and *A. variglandis* (Miller and Northup, 1926) are separate species, both probably in the genus *Austrobilharzia*. To further complicate the picture, differences between the Hawaiian schistosome as described by Chu and Cutress and *A. variglandis* according to Stunkard and Hinch-

liffe suggests that two distinct species may be involved.

The only other species allocated to the genus *Austrobilharzia* are *A. bayensis* Tubangui, 1933, described from one male specimen, and one whose larva, *Cercaria littorinalinae* Penner, 1950, is the only stage described. Penner (1953) stated that the adult from experimentally infected birds belongs to the genus *Austrobilharzia.*

Critical evaluation of the above species and others assigned to the genus *Microbilharzia* is beyond the scope of the present paper. The Laurer's canal and eversible cirrus observed in *A. penneri* have not been reported hitherto in species of *Austrobilharzia,* although figure 3 of Stunkard and Hinchliffe (1952) suggests that the terminal male genitalia in *A. variglandis* are similar to those of *A. penneri.*

The cercaria of *A. penneri* differs from *C. littorinalinae* and from cercariae described for *A. variglandis* and *A. terrigalensis* (see Holliman, in press). The species of snail host of *A. penneri, Cerithidea scalariformis,* is also different from those of the above species. The intermediate host of *A. bayensis* is unknown.

Adults of *A. penneri* and of *A. terrigalensis,* as described by Baerup (1956), differ in several respects: the ovary of the latter usually lies across the equator; the testes are said to vary from 12 to 20; and the male genital pore is figured as midventral.

Adults of *A. penneri* differ from those of *A. variglandis* as described by Stunkard and Hinchliffe (1952) mainly in number of testes and in their position in the body. Stunkard and Hinchliffe described 18 to 20 testes in the anterior one-half of the body. In 36 of 38 specimens of *A. penneri* the testes extend posterior to the equator. Also, the ovary in *A. penneri* is more anteriorly situated than in the two specimens pictured by Stunkard and Hinchliffe (1952). Further data supporting separation of these two species is found in chromosome studies, results of which will be published elsewhere.

Adults of *A. variglandis* from Hawaii, as described by Chu and Cutress (1954), differ from *A. penneri* in having: a larger body size in both sexes; 13 to 23 testes, depending on age; the gynecophoric canal extending anterior to the acetabulum, pictured clearly different from that of *A. penneri*; a midventral male genital pore (their fig. 1); a postequatorial ovary (their fig. 4); and the intestinal cecum of the female overlapping the excretory vesicle.

When Penner (1953) suppressed the genus *Microbilharzia* he considered only *M. chapini* Price, 1929, the type, which he placed in synonomy with *Austrobilharzia variglandis.* Other described species of *Microbilharzia* are *M. canadensis* McLeod, 1936; *M. manitobensis* McLeod, 1936; *M. lari* (McLeod, 1937) McLeod, 1940; and *M. hoepplii* (Tang, 1951) Dutt and Srivastava, 1955. *A. penneri* differs from all species assigned to the genus *Microbilharzia* in possession of fewer testes and a more anteriorly situated ovary.

Literature Cited

Baerup, A. J. 1956 Life cycle of *Austrobilharzia terrigalensis* Johnston, 1917. Parasitology **46**: 470–479.

Chu, G. W. T. C. and C. E. Cutress 1954 *Austrobilharzia variglandis* (Miller and Northup, 1926) Penner, 1953, (Trematoda: Schistosomatidae) in Hawaii with notes on its biology. J. Parasit. **40**: 515–524.

Holliman, R. B. Trematode cercariae from the Apalachee Bay area, Florida. Tulane Studies in Zool. (In press.)

Penner, L. R. 1953a The red-breasted merganser as a natural avian host of the causative agent of clam diggers' itch. J. Parasit. **39** (supp): 20.

Penner, L. R. 1953b Experimental infections of avian hosts with *Cercaria littorinalinae* Penner, 1950. J. Parasit. **39** (supp.): 20.

Price, E. W. 1929 A synopsis of the trematode family Schistosomidae with descriptions of new genera and species. Proc. U. S. Natl. Mus. **75**: 1–39.

Stunkard, H. W. and M. C. Hinchliffe 1952 The morphology and life-history of *Microbilharzia variglandis* (Miller and Northup, 1926) Stunkard and Hinchliffe, 1951, avian blood-flukes whose larvae cause "swimmer's itch" of ocean beaches. J. Parasit. **38**: 248–265.

Explanation of Plates

All drawings were made with the aid of camera lucida, and from stained whole mounts except figures 8 and 9 which were drawn from living unfixed eggs. Scale A applies to figures 2, 3, 5; B to figures 1, 4; C to figures 6, 7, 10; D to figure 9; E to figures 11, 12, 13.

FIGURE 1. Male, ventral view.
FIGURE 2. Detail of cirrus pouch and contents.
FIGURE 3. Cirrus everted.
FIGURE 4. Female, ventral view.
FIGURE 5. Detail of posterior end of ovary, oviduct, seminal receptable and Laurer's canal.

FIGURE 6. Male, ventral view in region of acetabulum.
FIGURE 7. Anterior end of male, side view.
FIGURE 8. Egg with mature miracidium, from gut tissue.
FIGURE 9. Posterior ends of eggs showing different types of spines and knobs.
FIGURE 10. Testes, showing different shapes, dorsal and ventral views.
FIGURES 11–13. Posterior ends of males, showing variation in intestinal tract.

RESEARCH NOTE

SOME PARASITES OF MAN IN ETHIOPIA.

While engaged in clinical evaluations of some new drugs in Addis Ababa, Ethiopia, during the period March through June 1959, a total of 680 stool specimens from as many persons were examined for intestinal parasites. As there appear to be no records of surveys made of intestinal parasitism in this country, the data on incidence are tabulated and presented.

The work was conducted at the Empress Zauditu Memorial Hospital in Addis Ababa. Patients were referred by the hospital staff, but most of those examined were hospital personnel, such as kitchen help and other employees. In addition there were included institutionalized persons of a blind school and an orphanage. Nearly all of the persons examined were Ethiopians; about 5 percent were Europeans and Americans.

Fresh stool specimens were examined immediately or within 3 hours from the time received by the warm-saline-smear method, as well as by the iodine method. Concentrations were made by the merthiolate-iodine-formalin method (MIFC) in most of the formed stool specimens. A number of smears were also stained by the iron-hemotoxylin and the PVA-trichrome methods.

The number and percent of parasites found in the 680 stools examined are presented in table I. The smaller amebas were seen, but not consistently identified, and are, therefore omitted from the tabulation. Of the 112 *E. histolytica* infections seen, 4 stools contained both trophozoite and cyst stages, while 95 had only trophozoites and 21 had only cysts. Of *E. coli*, 91 of the 150 persons had the trophozoite stage and 80 the cyst stage, while 21 harbored both.

TABLE I. *Incidence of parasites in 680 fecal specimens examined in Addis Ababa, Ethiopia.*

Parasite	Positive specimens	
	Number	Percent
Entamoeba histolytica	112	16.5
Entamoeba coli	150	22.1
Giardia lamblia	32	4.7
Trichomonas hominis	35	5.2
Chilomastix mesnili	35	5.2
Trichuris trichiura	76	11.2
Strongyloides stercoralis	68	10.0
Ascaris lumbricoides	57	8.4
Hymenolepis nana	16	2.4
Hookworm	11	1.6
Schistosoma mansoni	4	0.6

Proglottids and eggs of *Taenia saginata* were seen in 10 persons (omitted from table). Many more persons demonstrated proglottids. Since no stool was provided in addition to the proglottids, these are not included in this tabulation.

Amebiasis is the most important parasitic disease in Addis Ababa and some outlying districts in Ethiopia. Those having the cyst and/or trophozoite stage had symptoms of the disease. Approximately half of those harboring the trophozoite stage had an acute infection with diarrhea or severe dysentery. This is in sharp contrast to the chronic type of infections seen in other parts of Africa (Kenya and Nyasaland, to be reported elsewhere), where symptomless carriers were the rule.

Grateful acknowledgement is made to Parke, Davis and Company who supported this and allied work.—EDWARD D. WAGNER, *Department of Microbiology, School of Medicine, College of Medical Evangelists, Loma Linda, Calif.*

PLATE I

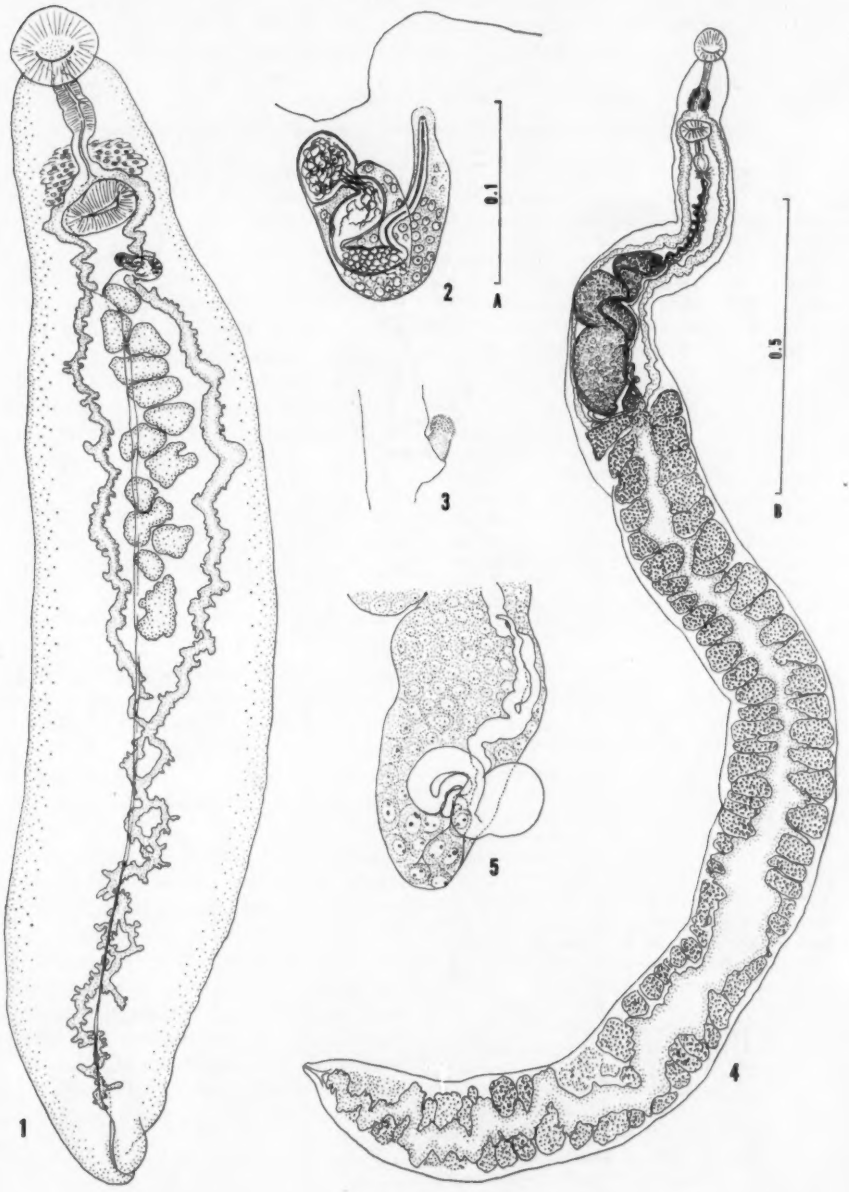
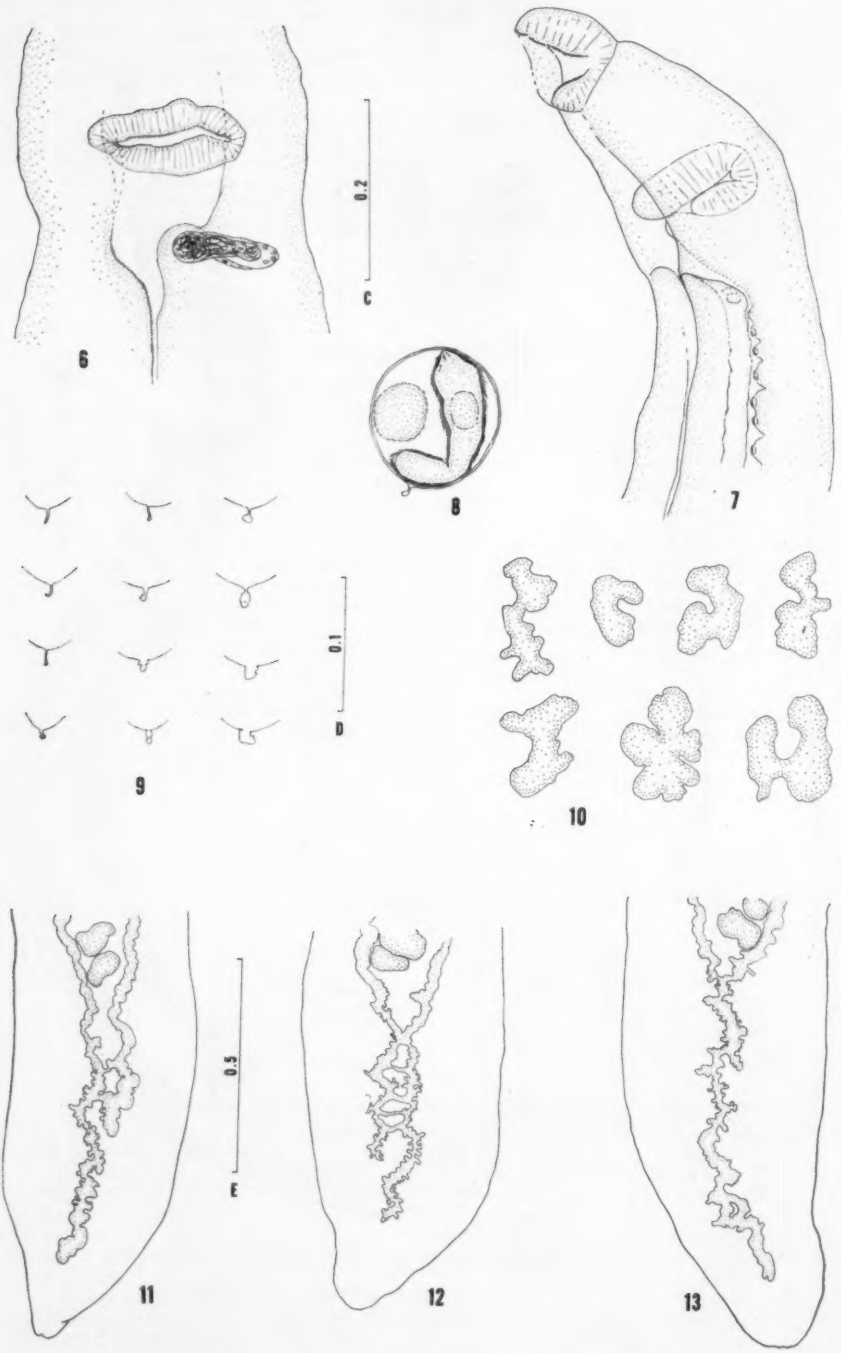


PLATE II



CONTRIBUTIONS TO THE LIFE HISTORY OF THE BRAIN FLUKE OF NEWTS AND FISH, *DIPLOSTOMULUM SCHEURINGI* HUGHES, 1929 (TREMATODA: DIPLOSTOMATIDAE)*

FRANK J. ETGES

Department of Biological Sciences, University of Cincinnati

In the summer of 1956, examination of 71 adults of the common newt, *Notophthalamus* (Syn. *Triturus*) *viridescens* (Raf.), collected in Mountain Lake, Virginia, revealed 100 percent incidence of a fluke (then tentatively identified as *Diplostomulum trituri* Kelley, 1934) infecting the brain and eyes. Attempts then made by the writer to obtain metacercariae or adults of the worm in laboratory mice and one-day-old chicks were unsuccessful, an experience suffered previously by T. C. Cheng and E. W. Lautenschlager (personal communication), who also worked on this problem at Mountain Lake.

In 1957 the writer began further investigations with a trematode survey of snails in Mountain Lake. Three strigeid infections were found among 107 specimens of *Helisoma anceps* (Menke), the cercariae of which were subsequently shown to be larvae of the brain fluke of newts. Careful study of existing descriptions of similar cercariae indicate that this cercaria has not been previously described. Infected snails had the peculiar thick shell structure noted by Etges (1961) in snails from this lake infected with an echinostome, *Cercaria reynoldsi*.

Due to difficulty in feeding heads or brains of *Notophthalamus* to mice and chicks, several dozen small specimens of *Lepomis gibbosus* (Linn.), *Lepomis* spp., and *Micropterus salmoides* (Lacepede) were collected to determine whether they would better serve as a transmission vehicle. The brain and eyes of these Mountain Lake fish, however, were already infected with diplostomula indistinguishable from those in newts. Questions of relation between the two forms, and their host specificity were thereby raised.

Received for publication December 3, 1960.

* This study was conducted at the Mountain Lake Biological Station, and supported by a National Science Foundation Grant-In-Aid awarded by the University of Virginia.

MATERIALS AND METHODS

Snails were isolated in vials to identify their infections, then marked with nail polish and maintained in aerated aquaria. Adult newts were kept in culture bowls in groups of three, larvae in groups of 15 to 25, and terrestrial red efts maintained in Petri dishes on wet filter paper. Fish were kept in shallow pans and culture bowls with gauze covers.

The following description is primarily based on living, naturally emergent cercariae, stained with neutral red. Refrigeration of cercariae for one to two hours was helpful in studying the excretory system, and prevented rapid breakdown of caudal setae or 'flagellets." Coverglass pressure was avoided by means of vaseline seals, which prevented evaporation and held the coverglass well above the slide. More than 50 permanently mounted cercariae were examined, and 38 studied alive. Camera lucida drawings were made of the former specimens, which were fixed in hot A.F.A., stained with Semichon's Acetic Acid Carmine, and mounted in Gum Damar. Fine details seen in live worms were drawn freehand.

Intramolluscan Stages of *Diplostomulum scheuringi*

(Dimensions given in millimeters)

Sporocysts (figs. 2a, b, c.): Typical unbranched, filiform strigeid larvae found in intercellular spaces of hepatopancreas and gonads of *Helisoma anceps;* prominent yellow pigment deposits in walls; non-adherent to host tissues; containing very large numbers of germ balls, developing or full-grown cercariae, or all three; birth pore, seen easily during cercarial emergence, subterminal; cercarial development morphologically complete at birth, behavior indistinguishable from naturally emergent cercariae; sporocyst body length, 0.450 to 3.00, diameter 0.050 to 0.310, small specimens tending to shorten and thicken more than older worms (figs. 2a and c); motility slow or absent; an estimated 30, 180, and 250 sporocysts were harbored by three snails, respectively. It is of interest that the snail with 30 sporocysts shed more cercariae daily than the one with 180.

Cercariae (figs. 1a, b): Of the non-oculate, longifurcate, strigeid type, with cylindrical, annulated bodies. Body, 0.182 to 0.266 long by 0.024 to 0.042 wide at mid-body, constricted into 16 to 18 prominent annulations, bearing complete rings of small posteriorly directed spines on annular crest; the most anterior annulus bears three closely set rows of spines, just behind oral opening; one

pair of prominent lateral flagellets or setae situated at level of excretory bladder; acetabulum circular, 0.015 to 0.021 in diameter, situated three-fourths of body length from anterior end, bearing two staggered rows of short spines around aperture; oral organ protrusible, terminal, 0.034 to 0.043 long by 0.018 to 0.028 wide; digestive system poorly differentiated, represented by a strand of small cells, extending posteriorly from oral organ, bifurcating near mid-body; pharynx very small, just behind oral organ; three pairs of post-acetabular cephalic glands, 0.007 to 0.014 in diameter lie in parallel longitudinal rows between acetabulum and excretory bladder, their ducts passing anteriorly on each side, dorsal to oral organ, to three pores on either side of mouth; two pairs of prominent, unidentified, granular bodies lie just anterior to acetabulum, characterized by their anucleate, ductless, dark-granular nature; genital primordium poorly differentiated, between cephalic glands and bladder, almost completely obscured by primordium of ventral holdfast organ; excretory bladder, subspherical with heavy walls, receiving two lateral ducts from body, and one medial duct from tail in which a proximal bladder is formed by dilation of caudal duct; flame cells and ducts in body and tail as shown in figure 1a, described by the formula $2[(3+2)+(2+3)]$; excretory pore ventral, subterminal, served by short duct containing an "island of Cort"; tail stem cylindrical, finely annulated, almost as long as body, 0.148 to 0.232 long by 0.020 to 0.031 wide, bearing ten pairs of lateral flagellets proximally and ten more pairs distally; length of tail setae, 0.011 to 0.017; caudal bodies small, numerous, situated along contractile medullary cord and excretory duct; caudal excretory bladder leads posteriorly through a bifurcated duct to subterminal pores on leading edges of furcae, connects anteriorly to primary bladder of body; furcae, slightly flattened, 0.160 to 0.191 long, lacking cuticular spines, setae, or finfolds.

More than 200 cercariae emerge from one snail in a 24-hour period, mainly between 6:00 and 11:00 a.m. No evidence of phototaxis was noted, but mechanical stimuli caused increased activity of cercariae. Lying on the bottom or drifting freely, the body continuously flexes and extends, often assuming the characteristic form shown in figure 1b. Attachment to surface film or surface of container is rare.

Among previously described cercariae, a small number are of the distome, non-oculate, longifurcate, cylindrically annulated, strigeid type, possessing three pair of post-acetabular cephalic glands, caudal setae, and small numerous caudal bodies, as found in the cercaria of *Diplostomulum scheuringi*. A further distinctive feature of this species, a poorly developed digestive system, is similar to that described by Hoffman (1958) for cercariae of *Ornithodiplostomulum ptychocheilus*, an oculate species similar to *Posthodiplostomum minimum centrarchi* described by Miller (1954). The following cercariae are those from which the present species need be distinguished here: *Cercaria emarginatae* Cort, 1917, as redescribed by Olivier and Cort (1942), *C. wardlei* McLeod, 1934, *C. friedricksborgensis* Wesenberg-Lund, 1934, *C. I.* Petersen, 1931, as redescribed by Wesenberg-Lund (1934), *C. dohema* Cort and Brackett, 1937, *C. murrayensis* Johnston and Cleland, 1938, *C. riponi* Brackett, 1939, *C. wallooni* Olivier, 1941, *C. higginsi* Olivier, 1942, and *C. angelae* Johnston and Simpson, 1944. All of these species are distinct from the present cercaria in possessing well-developed digestive systems. All except *C. wallooni* and *C. murrayensis* have very large caudal bodies, not seen in *C. D. scheuringi*. *C. wallooni* differs from the present species in flame cell number and location, bearing spines on the caudal furcae, having 10 body annulations and non-linear arrangement of cephalic glands. *C. wardlei* and *C. murrayensis* both possess a distinctive caudal flexure and constriction of the body not found in the present form. Further, Johnston and Simpson (1939) demonstrated that *C. murrayensis* develops into diplostomula markedly different from *D. scheuringi*. *C. angelae* differs from the present species in possessing four pair of cephalic glands, a collar of eight rows of short spines, and lacking body and tail setae. *C. higginsi* differs in lacking flagellets, having 12 body annuli, and its cephalic glands clustered rather than linear. *C. emarginatae* has ovoid body form, six pair of flame cells, and was shown experimentally by Olivier and Cort (1942) to develop into a diplostomulum easily distinguished from *D. scheuringi*. *C. riponi* has unpigmented eyespots, clustered cephalic glands, and cuticular spines only on the anterior third of its non-annulated body. *C. I* also lacks body annulations. *C. friedricksborgensis* has an ovoid, non-annulated body. In possessing clustered cephalic glands, four pair of flame cells, and well-developed digestive system and caudal bodies, *C. dohema* clearly differs from *D. scheuringi*. In addition to the qualitative differences cited above, numerous differences of size and proportion exist between these species and cercariae of *D. scheuringi*.

EXPERIMENTAL INFECTION OF VERTEBRATES

Initially, experiments were conducted by ex-

posing adult newts, *Notophthalamus viridescens*, to the total 24-hour cercarial output of one snail. In 26 attempts to infect adult newts, cercariae were seen to crawl about on the skin, but apparently unable to penetrate, soon swam away. Some cercariae which reached the newt's eye, however, shed their tails and began vigorous "digging" movement with the oral organ. Within 12 minutes cercariae entered the anterior eye chamber, having penetrated the cornea without discomfort to the newt. Daily autopsy of 14 newts revealed numerous active cercarial bodies in the eye humours, but no invasion of retina, lens, or optic nerve. In this time worms underwent 2 to 3-fold growth in size, but no differentiation of new structure. Prior to this experiment, newts were isolated for 24 days to allow growth of worms already present, making distinction of natural and experimental worms possible by size difference. Since only four of 24 newts had natural ocular infections, difficulty in this respect was practically eliminated. Results of this experiment only establish that cercariae can penetrate and develop in the eye of adult newts. Subsequent experiments established that this worm can also enter the eyes of newt larvae, red efts, and various small fish (*Lepomis gibbosus, Lepomis* spp., *Micropterus salmoides,* and unidentified minnows collected in Sinking Creek, Virginia). Adult *Pseudotriton* sp., and tadpoles and adults of *Rana palustris* and *R. pipiens* were found impervious to penetration by this cercaria. Further, no natural infection with *D. scheuringi* has been found in frogs and salamanders collected in Mountain Lake. Whether brain and ocular infections found in newts and fish were due to the same cercaria, and the means by which brain vesicles were reached, remained to be determined.

Since the adult newt's skin is apparently refractory to cercarial penetration, it seemed likely that newt larvae would permit cutaneous entry of worms which could then reach the brain through circulation. Few larvae of *Notophthalamus* in Mountain Lake were found infected with diplostomula in the brain (7 of 61 autopsied); no ocular infection was seen. Exposure of 10 newt larvae to massive cercarial penetration killed them within 2 hours; autopsy revealed numerous cercarial bodies in the brain, eye, liver, spleen, pancreas, intestinal wall, body cavity, and tail muscle. Reducing the number of cercariae to about 100 reduced host mortality, but pronounced discomfort was still indicated by violent movements of the larvae during one hour of exposure. Studies on 48 newt larvae over a 20-day period showed that cercariae penetrate the skin at any point, cause small subcutaneous hemorrhages, migrate via circulation to all parts of the body, then gradually accumulate in the brain vesicles. By the sixth day no worms were found outside the brain and eyes. Growth of unencysted worms is rapid, increasing their volume 10-fold by the 20th day, and development of the posterior hold-fast organ is clear (compare figures 3a and 3b).

During the 20-day period of this experiment, several newt larvae metamorphosed to the terrestrial red eft stage. Exposure of efts to cercariae indicated a pronounced increase in resistance to cutaneous penetration, while the cornea remained susceptible. Most cercariae soon swim away from the skin, after an abortive attempt to penetrate, but several worms entered successfully. Apparently *Notophthalamus* is most vulnerable to infection in its larval stage, but remains susceptible for most of its life via the ocular route. The writer did not attempt to determine whether cercariae can migrate from the eye to the brain through the optic stalk, but judging from the low prevalence of natural brain infections in larvae and fully developed red efts, contrasted with universal occurrence in adult newts, it appears that such migration is probable.

Experiments using small fish (named above) resulted in equally conclusive observations of penetration, irritation, cutaneous hemorrhage, migration, and localization of diplostomula in eyes and brain. Cercariae used in these experiments emerged from the same three snails used in amphibian experiments. Dissection of the snails revealed that each was infected with the same species of strigeid cercaria. Therefore, this fluke has little apparent specificity for the second intermediate host, penetrating and developing in both fish and newts.

Growth and development of experimentally introduced diplostomula to definitive size occurred in two minnows collected in Sinking Creek, more than 5 miles from Mountain Lake, and two red efts which had developed from larvae collected in Mountain Lake. Figure 4a shows a mature diplostomulum from the minnow brain, figure 4b shows a worm from the

red eft's brain, found 45 and 48 days after exposure to cercariae, respectively. In size and structure, both worms are indistinguishable from descriptions of *D. scheuringi* given by Hughes (1929), Van Cleave and Mueller (1934), Chandler (1951), and Haderlie (1953), *D. trituri* given by Kelley (1934), and *Diplostomulum* sp. given by Lautenschlager (1956). Comparison of the structure of naturally occurring diplostomula in newts and fish, from both eye and brain, together with experimental demonstration of their common origin, convinces the writer that they are the same. Accordingly, *Diplostomulum trituri* Kelley, 1934, is suppressed as a synonym of *Diplostomulum scheuringi* Hughes, 1929. It is likely that the species studied by Lautenschlager (1956) is also synonymous with *D. scheuringi,* but since no study of brain pathology of the present material was made, it remains possible that two morphologically indistinguishable species could exist, one of which causes damage as described by Lautenschlager, the other being non-pathogenic in newts.

Since large numbers of *D. scheuringi* were available, several attempts were made in this study to obtain the encysted larva or adult stage. Hundreds of fully grown diplostomula from both newts and fish fed to 22 one-day old chicks gave no evidence of encystment or sexual maturation. In 31 mice, infections were absent following oral administration of diplostomula. In one chick and one mouse, autopsied within 2 hours of feeding, dead bodies of several worms were found in the upper small intestine. One mouse, which had received more than 50 worms from one newt, had three encysted worms among the axillary muscles. These worms encysted in warm saline, and except for slight growth of the hind-body are seen (fig. 5) to be identical to specimens from the newt brain. The worms in this case were administered by a syringe which was accidentally inserted into the trachea rather than the esophagus, and this mishap apparently allowed survival of these few worms. A similar observation was made by Odlaug (1940) who found that mesocercariae of *Alaria intermedia* could be successfully introduced into the pleural cavity of mice through a small thoracic incision. The worms are usually destroyed when fed to mice, according to Odlaug.

The significance of encystation by *Diplstomulum scheuringi* is problematic with respect to determining the identity of a third intermediate host. Since the worms were unable to survive digestion by chicks and mice, yet possess capacity to encyst in mice, it is suggested that an encysted stage may develop in a cold-blooded host, perhaps snakes, and that the entire life cycle probably involves four obligate hosts. The cysts produced in a mouse in the present study are regarded as aberrant by the writer, probably due to the experimental accident described above. Until the life history of this worm is completed, it is not possible to make a precise generic allocation for it. It will also be of considerable interest to determine the relative importance of newts and fish as natural vectors of *Diplostomulum scheuringi.*

SUMMARY AND CONCLUSIONS

The intramolluscan stages of *Diplostomulum scheuringi* are identified and described. The snail host was found to be *Helisoma anceps* (Menke).

In addition to all stages in the life history of the newt, *Notophthalamus viridescens,* this cercaria is experimentally shown to penetrate and develop in the eye and brain of various species of fish.

In view of the identical structure of naturally occurring diplostomula in newts and fishes, and the experimental demonstration of common origin, *Diplostomulum trituri* Kelley, 1934, is suppressed as a synonym of *Diplostomulum scheuringi* Hughes, 1929.

The existence of a four-host life cycle is suggested for *D. scheuringi,* based on the demonstration of its capacity to encyst in mice and its inability to survive digestion in chicks and mice.

ACKNOWLEDGMENT

The writer here expresses his appreciation to Dr. J. P. E. Morrison, Division of Mollusks, The Smithsonian Institution, for his identification of the snail species involved in the present study.

REFERENCES

CHANDLER, A. C. 1951 Studies on the metacercariae of *Perca flavescens* in Lake Itasca, Minnesota. Am. Midl. Nat. **45**: 711–721.

ETGES, F. J. 1961 *Cercaria reynoldsi* n. sp. (Trematoda: Echinostomatoidea) from *Helisoma anceps* (Menke) in Mountain Lake, Virginia. Tr. Am. Micr. Soc. 80. In Press.

HADERLIE, E. E. 1953 Parasites of the freshwater fishes of California. Univ. Calif. Publ. Zool. **57**: 303–440.

HOFFMAN, G. L. 1958 Studies on the life cycle of *Ornithodiplostomulum ptychocheilus* (Faust) (Trematoda: Strigeoidea) and the "self-cure" of infected fish. J. Parasit. **44**: 416–421.

HUGHES, R. C. 1929 Studies on the trematode family Strigeidae (Holostomidae) No. XIX. *Diplostomulum scheuringi* sp. nov. and *D. vegrandis* (La Rue). J. Parasit. **15**: 267–271.

JOHNSON, T. H. AND SIMPSON, E. R. 1939 The diplostomulum stage of *Cercaria murrayensis*. Tr. Roy. Soc. Austral. **63**: 230–237.

——— AND ——— 1944 Larval trematodes from Australian freshwater molluscs. Part IX. Tr. Roy. Soc. S. Austral. **68**: 125–132.

KELLEY, R. S. 1934 The trematode parasites of *Triturus v. viridescens* (Raf.). Univ Pitt. Bull. **31**: 201–210.

LAUTENSCHLAGER, E. W. 1956 A study of *Diplostomulum* sp. (Trematoda: Diplostomatidae) in the brain of *Triturus v. viridescens* (Rafinesque). M.S. Thesis (Unpublished) University of Virginia, Dept. of Biology. 27 pp.

MILLER, J. H. 1954 Studies on the life history of *Posthodiplostomum minimum* (MacCallum, 1921). J. Parasit. **40**: 255–270.

ODLAUG, T. O. 1940 Morphology and life history of the trematode, *Alaria intermedia*. Tr. Am. Micr. Soc. **59**: 490–510.

OLIVIER, L. AND CORT, W. W. 1942 An experimental test of the life cycle described for *Cotylurus communis* (Hughes). J. Parasit. **28**: 75–81.

VAN CLEAVE, H. J. AND MUELLER, J. F. 1934 Parasites of Oneida Lake fishes. Part III. A biological and ecological survey of the worm parasites. Roosevelt Wildlife Ann. **3**: 160–334.

WESENBERG-LUND, C. 1934 Contributions to the development of the Trematoda Digenea. Part II. The biology of the freshwater cercariae in Danish freshwaters. Danske Vidensk. Selsk. Skr. Naturv. og Math. Afd. **5**: 1–223.

EXPLANATION OF PLATE

All drawings were made with the aid of camera lucida, and details were added freehand unless otherwise noted. Scales given in millimeters. All figures are of *Diplostomulum scheuringi* Hughes, 1929.

FIGURE 1a. Details of cercarial structure, showing excretory ducts and flame cells only on one side, cephalic glands, and ducts on the other.

1b. Lateral aspect of cercaria, showing body form while floating in water, excretory bladder and ducts, and cephalic glands and ducts; freehand sketch.

FIGURE 2a. Fully grown sporocyst containing germ balls and cercariae in stages of development.

2b. Young sporocyst, lacking birthpore, containing germ balls and developing cercariae.

2c. Partially developed sporocyst, showing birthpore.

FIGURE 3a. Diplostomula from brain of experimentally infected newt larva, 20 days after cercarial penetration.

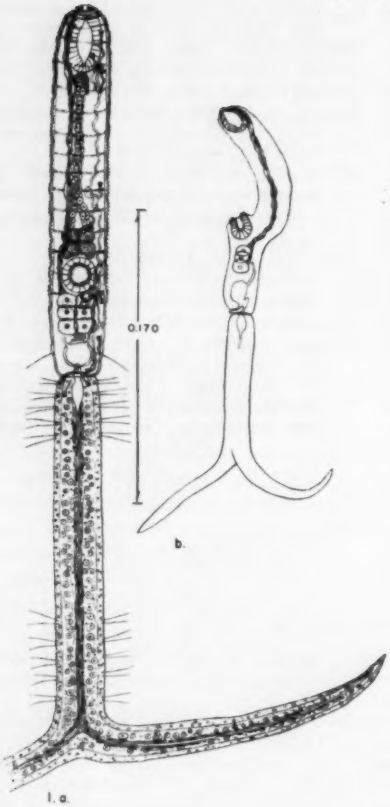
3b. Cercarial body from brain of experimentally infected newt larva, 3 days after cercarial penetration.

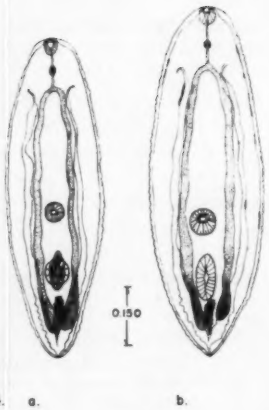
FIGURE 4a. Fully developed, unencysted mesocercaria from brain of minnow, 45 days after experimental infection.

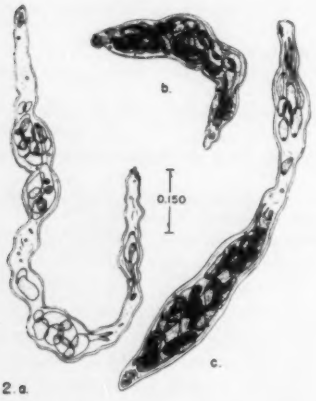
4b. Fully developed, unencysted mesocercaria from brain of experimentally infected red eft stage of the newt, 48 days after cercarial penetration.

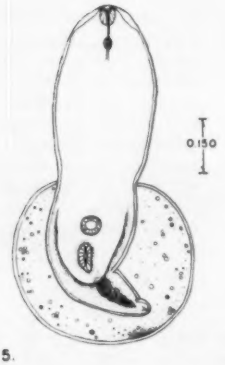
FIGURE 5. Encysting worm from experimentally infected mouse, 6 days following introduction into the lung.

PLATE I









HISTOCHEMICAL GLYCOGEN STUDIES ON *FASCIOLA HEPATICA*

THEODOR VON BRAND AND TERESA I. MERCADO

U. S. Department of Health, Education, and Welfare, Public Health Service,
National Institutes of Health, National Institute of Allergy and Infectious Diseases,
Laboratory of Parasitic Diseases, Bethesda, Maryland

Fasciola hepatica, like many other parasitic helminths, has a fermentative type of carbohydrate utilization which leads to a rapid turnover of carbohydrate. When starving in vitro it utilizes in 5 hours approximately 20 percent of its glycogen reserve (Weinland and von Brand, 1926). When starved over night, approximately 50 percent of its glycogen stores disappear (Mansour, 1959). These quantitative observations suggested that a histochemical study could reveal from what organs the polysaccharide was disappearing primarily. The anatomical peculiarities of flatworms preclude the use of isolated organs for quantative studies. In view of their large glycogen reserves the liver flukes, on the other hand, are especially suited to histochemical polysaccharide investigations. The results of our relevant studies are summarized below.

MATERIAL AND METHODS

The *Fasciola* specimens,** obtained from the bile ducts of cattle, were divided into four groups, each consisting of six to eight worms. Group 1 was represented by newly isolated organisms, while they had been starved for 12 and 24 hours respectively in groups 2 and 3 respectively. During the period of inanition the worms were kept at 37.5 C in the saline solution recommended by Dawes (1954) for the maintenance of liver flukes. The animals of group 4 were first starved for 24 hours and then kept for another 24 hours in a medium containing glucose and 30 percent serum, that is, under conditions that according to Mansour's (1959) quantitative observations lead to a rapid resynthesis of polysaccharide. All incubations were done in the presence of penicillin (2,000 units per ml) and streptomycin (0.1 mg per ml).

The worms were fixed in Carnoy's solution and embedded in paraffin. Sections, 7 microns thick, were stained for polysaccharide by the periodic acid Schiff procedure and counterstained with Harris' haematoxylin. Control slides digested previously with diastase were stained similarly and served to establish the glycogen nature of the polysaccharide studied.

RESULTS AND DISCUSSION

The intimate glycogen distribution in the tissues of *Fasciola* specimens recently isolated from the host has been described several times, in greatest detail by Ortner-Schoenbach (1913) and Axmann (1947). Our findings on freshly isolated flukes are in full accord with the observations of these authors. It suffices therefore to summarize them very briefly and to mention only those organ systems which serve to store most of the morphologically demonstrable polysaccharide. The parenchymal cells were filled with glycogen granules of various sizes in all regions of the worms and probably should be considered as the most important storage place. Large amounts of polysaccharide occurred also in the muscular organs, especially the suckers and the cirrus pouch. The glycogen is not deposited within the contractile parts of the muscle cells, but within the non-contractile parts and especially within the parenchymal sheath of the cells, as especially Ortner-Schoenbach (1913) emphasized. The ovarian eggs contained some glycogen, but most of the polysaccharide localized in the female reproductive system was found in the mature vitellarian cells and the uterine ova.

After 12 hours' starvation some glycogen depletion could be discerned with the parenchyma. However, there was some variation among various specimens and the changes were not pronounced enough to be entirely convincing in all cases. No change in glycogen content of the muscular organs could be seen, as a comparison of plate I, figures 1 and 2 indicates. After 24 hours' starvation, on the other hand, the histochemical polysaccharide picture had changed radically. The parenchyma was almost completely devoid of glycogen as the contrast of

Received for publication December 20, 1960.

** We are indebted to Dr. Tag E. Mansour for isolating, maintaining and fixing the flukes used in this study.

figures 1 and 5 (plate II) and figures 2 and 6 (plate II) indicates. Similarly, the muscular organs had lost most of their polysaccharide (see plate I, figure 3 on the one hand, and plate I, figures 1 and 2 on the other). On the contrary, the glycogen content of the vitellaria appeared essentially unchanged regardless of the length of inanition (plate II, figures 1, 2, and 3) and the same applies to the uterine eggs.

It is of course probable that the apparently dramatic decrease in histochemically demonstrable polysaccharide during the 12- to 24-hour starvation period, as opposed to the hardly demonstrable decrease during the initial 12-hour period of inanition, is due to the fact that even a quantitatively marked glycogen disappearance is not readily demonstrated histochemically in an organ initially very rich in polysaccharide. The overall glycogen concentration in freshly isolated *Fasciola* specimens lies between 3 and 4 percent of the fresh tissues, corresponding to about 15 to 20 percent of the dried tissues (Flury and Leeb, 1926; Weinland and von Brand, 1926; Mansour, 1959). The polysaccharide concentration within the parenchymal cells undoubtedly is considerably higher than the overall figure indicates, but no exact data are available. On the whole it does appear probable that the parenchymal glycogen is mobilized slightly faster than that present in the muscular organs. A similar polysaccharide utilization though apparently differing in the relative rapidity of parenchymal and muscular glycogen disappearance has been reported by Dawes and Muller (1957) in the case of *Haplometra cylindracea* starving in vitro. We wish also to emphasize that, although we could not demonstrate histochemically a glycogen disappearance from the vitellarian cells, we do not exclude the possibility that some glycogen localized within these cells may have been utilized.

Flukes starved for 24 hours and then refed had, as far as can be judged from histochemical demonstration, resynthesized the lost carbohydrate completely. Some specimens even gave the impression of having a higher polysaccharide concentration in parenchyma and muscles than freshly isolated ones (compare figures 1 and 4 of plate I and figures 1 and 2 of plate II.) Mansour's (1959) quantitative determinations had shown an approximately complete overall resynthesis of the lost polysaccharide under conditions analogous to those employed in the present studies.

Orrell and Bueding (1958) described for *Ascaris* a polydispersity of glycogen characterized by two regions of sedimentation coefficients. More recently (personal communication) they found a similar polydispersity in the case of *Fasciola* and they observed that in starved specimens the heavier component disappeared almost completely while the lighter one was still demonstrable. It is then tempting to correlate our histochemical findings with these observations. This, however, can be done only with caution and tentatively because of the limitations inherent in demonstration of histochemical glycogen disappearance. Essentially, only one conclusion appears warranted: The glycogen remaining in flukes after 24 hours' starvation and situated primarily within the vitellarian cells and the uterine eggs must be of the low molecular variety. We wish to emphasize that the present observations do not throw any light on the question whether, in well fed flukes, the above structures contain both the low molecular variety and the high molecular variety, or whether the latter is located primarily elsewhere (e.g., parenchyma, muscular organs). Similarly, our observations do not show whether the organs primarily involved in glycogen depletion (parenchyma and muscular organs) contained only one of the glycogen varieties, or a mixture of both.

SUMMARY

Histochemical studies show that during starvation the glycogen of *Fasciola hepatica* disappears primarily from the parenchymal cells and the muscular organs; while, at least during starvation periods of 12 or 24 hours' duration, no glycogen decrease could be observed in the vitellarian cells and the uterine ova.

REFERENCES

AXMANN, C. 1947 Morphological studies on glycogen deposition in schistosomes and other flukes. J. Morph. **80**: 321–334.

DAWES, B. 1954 Maintenance *in vitro* of *Fasciola hepatica*. Nature **174**: 654.

DAWES, B. AND MULLER, R. 1957. Maintenance *in vitro* of *Haplometra cylindracea*. Nature **180**: 1217.

FLURY, F. AND LEEB, F. 1926 Zur Chemie und Toxikologie der Distomen (Leberegal). Klin. Wochensch. **5**: 2054–2055.

MANSOUR, T. E. 1959 Studies on the carbohydrate metabolism of the liver fluke *Fasciola hepatica*. Biochim. Biophys. Acta **34**: 456–464.

ORRELL, S. A. AND BUEDING, E. 1958 Sedimentation characteristics of glycogen. J. Am. Chem. Soc. **80**: 3800.

ORTNER-SCHOENBACH, P. 1913 Zur Morphologie des Glykogens bei Trematoden und Cestoden. Arch. Zellforsch. **11**: 413–449.

WEINLAND, E. AND VON BRAND, T. 1926 Beobachtungen an *Fasicola hepatica* (Stoffwechsel und Lebensweise). Ztschr. Vergl. Physiol. **4**: 212–285.

RESEARCH NOTE

SIZE OF ADULT *NIPPOSTRONGYLUS BRASILIENSIS* FROM LIGHT AND HEAVY INFECTIONS IN LABORATORY RATS

Several investigators have observed a crowding effect in cestode infections in which the size of the individuals decreased with increasing numbers of worms (Read, 1951, J. Parasit. **37**: 174–178). Little is known about whether such a phenomenon exists in nematode infections. Ackert, Graham, Nolf and Porter (1931, Trans. Am. Micr. Soc. **50**: 206–214) reported that the length of *Ascaridia galli* was greater in chicken given 25 to 50 eggs than in those given 100 to 500 eggs. It is interesting, however, that there was little difference in the total numbers of worms recovered from animals given these different doses. The differences in worm length were attributed to a serological factor.

Recently, the writers had an opportunity to measure adults of the rat nematode, *Nippostrongylus brasiliensis* (Travassos, 1914) from light and heavy infections of laboratory rats. Living worms were removed from the small intestine of the hosts and placed in 0.9 percent saline. They were transferred, a few at a time, to a piece of saline-moistened filter paper on a glass plate under a dissecting microscope. With the assistance of a fine needle each worm was teased gently until straight, and its total length was measured with the aid of a dissecting microscope equipped with an ocular micrometer. The degree of moistness of the filter paper was found to be critical; if too much fluid was present the worms tended to float and could not be straightened for measurement. Fresh filter paper was used as needed.

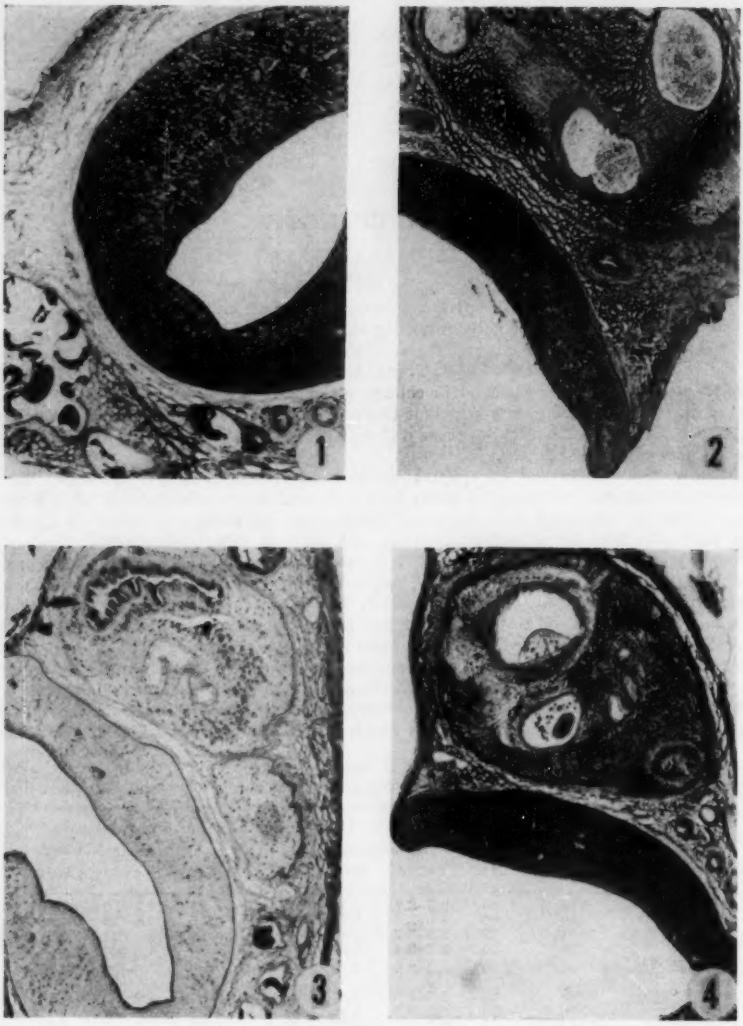
The lengths of 25 male and 25 female worms from each of 4 rats (Sprague-Dawley) with worm burdens ranging from 190 to 361 were compared with similar measurements from 4 rats that harbored from 1710 to 2290 worms. No differences were found between the lengths of the worms from the light and heavy infections (table I). It was concluded from these data that

TABLE I. *Length of adult male and female* N. brasiliensis *recovered from rats with small and large adult worm populations 10 days after intracutaneous inoculation with filariform larvae.*

Rat No.	Sex	No. larvae given	No. adult worms recovered	Length of adult worms in mm							
				Male worms				Female worms			
				No.	Range	Mean	S.D.	No.	Range	Mean	S.D.
1	M	450	196	25	2.5–3.7	3.15	0.27	25	4.1–5.2	4.68	0.30
2	M		190	25	3.0–3.8	3.39	0.20	25	3.6–5.2	4.80	0.31
3	F		361	25	2.8–3.7	3.19	0.25	25	4.1–5.1	4.62	0.23
4	F		270	25	2.2–3.4	3.01	0.31	25	4.0–5.1	4.48	0.27
Totals or Means			254	100	2.2–3.8	3.18		100	3.6–5.2	4.65	
5	M	3300	1988	25	2.5–3.9	3.21	0.36	25	4.0–5.2	4.65	0.33
6	M		2290	25	2.9–3.7	3.39	0.24	25	4.4–5.2	4.73	0.31
7	F		1710	25	2.3–3.6	3.03	0.28	25	3.8–5.1	4.44	0.30
8	F		2260	25	3.0–3.5	3.27	0.17	25	4.0–5.1	4.64	0.28
Totals or Means			2062	100	2.3–3.9	3.23		100	3.8–5.2	4.61	

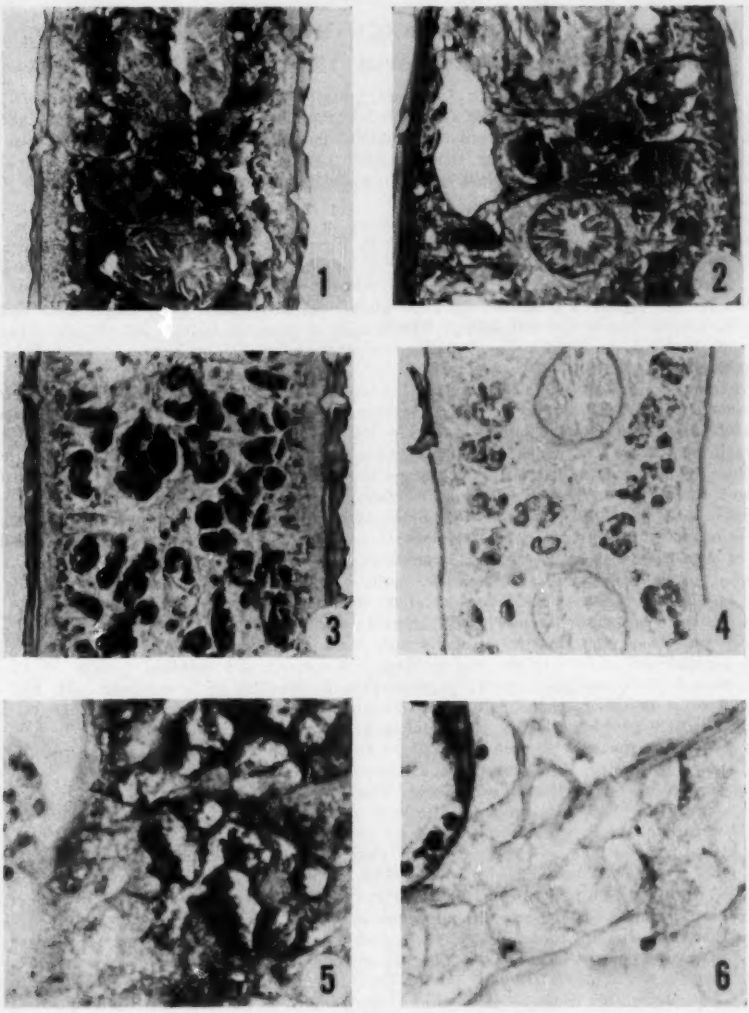
a crowding effect phenomenon does not exist in *N. brasiliensis* infections of laboratory rats. This may be correlated with the belief that the adults of *N. brasiliensis* are tissue feeders and that the host's gut contents are relatively unimportant in their diet (Rogers and Lazarus, 1949, Parasitology **39**: 245–250). (Supported by the General Research Board, Graduate School, University of Maryland.)—A. J. HALEY AND J. C. PARKER, *Zoology Department, University of Maryland, College Park, Maryland.*

PLATE I



Sections through region of ventral sucker of *Fasciola hepatica*, stained with PAS for glycogen and counterstained with Harris hæmatoxylin, × 63. Fig. 1, freshly isolated fluke; fig. 2, fluke starved for 12 hours; fig. 3, fluke starved for 24 hours (note almost complete disappearance of glycogen); fig. 4, fluke starved for 24 hours and kept for another 24 hours in a nutritive medium (note resynthesis of abundant glycogen).

PLATE II



Sections through middle part of body of *Fasciola hepatica*, stained as in plate I. Figs. 1 to 4, ×143; figs. 5 and 6, ×415. Fig. 1, freshly isolated fluke; fig. 2, fluke starved for 24 and kept for another 24 hours in a nutritive medium; fig. 3, fluke starved 24 hours; fig. 4, fluke starved for 24 hours. The slide from which fig. 4 was taken had been digested prior to staining, with diastase, while all other photographs are from undigested slides. Comparison of fig. 4 with figs. 1 to 3 indicates the amount of glycogen present in the vitellaria. Fig. 5, freshly isolated fluke; fig. 6, fluke starved for 24 hours. Comparison of these two figures shows the high degree of glycogen depletion taking place during a 24-hour starvation period.

RESEARCH NOTE

IS *BACILLUS PINOTTII* PATHOGENIC IN *AUSTRALORBIS GLABRATUS?*

Dias reported (1954, Proc. 6th Internatl. Cong. Trop. Med. and Mal.; Mem. Inst. Osw. Cruz **52**: 315–327) that *Bacillus pinottii*, isolated from *Australorbis glabratus*, is useful in the biological control of snails. Since only uncontrolled field studies had been conducted heretofore (Dias and Dawood 1955 Mem. Inst. Osw. Cruz **53**: 13–29; Texera and Scorza, 1954, Arch. Ven. Patol. Trop. Parasit. Med. **2**: 235–242), we attempted to confirm the pathogenicity of *B. pinottii* for *A. glabratus* in the laboratory.

The strain of *B. pinottii* used was obtained in 1954 by Dr. E. Chernin from Dr. C. B. Philip, who had just procured it from Dias in Egypt. It was subcultured on nutrient agar every few weeks from March to June 1954; it was then subcultured 1 to 2 times each year with interim storage at 5 C until April 1959, when it was studied bacteriologically and found similar to the organism described by Cruz and Dias (1953, Roy. Soc. Trop. Med. Hyg. **47**: 581–2), except that it did not change litmus milk or produce indol. All *A. glabratus* used were of the PR-1 strain maintained in this laboratory since 1954 (Chernin et al, 1956, Am. J. Trop. Med. Hyg. **5**: 297–307).

Attempts were made to determine the infectiousness of peptone-cultured (vegetative) *B. pinottii* for *A. glabratus* by repeating an experimental protocol of Dias' (1954). Snails were kept at 27 ± 2 C, in 250-ml flasks containing 100 ml of various dilutions of peptone (1 : 100 through 1 : 102,400) to which 1 ml of a 48-hour peptone culture of *B. pinottii*, or 1 ml of sterile peptone, was added. In peptone 1 : 100 through 1 : 1600, whether *B. pinotti* was present or not, all snails died within a few days, probably as a result of gross fouling of the medium. In higher dilutions snails died irregularly over a period of 1 month. These findings are in contrast to those of Dias (1954) who noted that snails in peptone plus *B. pinottii* died sooner than those in peptone alone. Tissue sections of experimental snails disclosed generalized atrophy, probably due to starvation. No pathology attributable to bacteria was noted, nor were any bacteria seen. Control snails (unfed) in flasks containing distilled or aquarium water lived 1 month and showed similar histologic changes.

In subsequent experiments all snails were kept in 2.5-liter jars and fed lettuce; all jars were aerated and covered with "Saran Wrap." Measured and matched groups of 15 snails each were kept in five dilutions of peptone plus *B. pinottii*, or in peptone only, and equal inocula of a 48-hour peptone culture of *B. pinottii* were added to the experimental series. In the 1 : 1000 peptone dilution gross fouling killed all snails within 48 hours whether or not *B. pinottii* was present. Several snails in 1 : 5000 peptone died during initial pollution and survivors grew poorly. At lower peptone concentrations (1 : 10,000, 25,000 and 100,000) all snails survived, grew well and reproduced. Tissue sections of survivors failed to reveal any pathology or bacteria. Analogous results were obtained in subsequent experiments in which snails were exposed to washed vegetative *B. pinottii*.

Attempts were made to find pathologic changes in *A. glabratus* following parenteral injection of living *B. pinottii*. Thrice washed 48-hour peptone cultures were suspended in sterile distilled water (0.1 ml packed cells to 10 ml) and 0.05 to 0.10 ml was injected into the cephalopedal sinus using a syringe and 30-gauge needle (Michelson, 1958, Trans. Am. Micr. Soc. **77**: 316–319). It was shown that: (1) viable *B. pinottii* could be recovered from injected snails at 2 and 48 hours, but not later; (2) *B. pinottii*-injected snails were normal, as compared to distilled water injected controls, in respect to survival and growth over a 10-week period; and (3) bacilli were detectable in tissue sections of snails fixed at 10 minutes and 1 hour post-injection, but not later.

The foregoing results indicate that our strain of *B. pinottii* is not pathogenic for *A. glabratus* either by direct exposure or by parenteral injection. Furthermore, earlier unpublished experiments done upon receipt of the organism in 1954 (Chernin and Michelson, 1954, personal communication) gave similarly negative results. Unfortunately, no other strain of *B. pinottii* is currently available for comparison (Dias, 1959, personal communication).

This study was supported (in part) by a Training Grant (No. 2E-46) from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, Public Health Service. M. R. TRIPP, *Department of Tropical Public Health, Harvard School of Public Health, Boston, Mass.*
(Present address: *Department of Biological Sciences, University of Delaware, Newark*)

A NEW SPECIES OF *HYSTEROLECITHA* (TREMATODA: HEMIURIDAE) FROM THE MUDSUCKER *GILLICHTHYS MIRABILIS* COOPER*

ROY E. KING AND ELMER R. NOBLE
University of California, Santa Barbara

During the course of a four-year investigation of the estuarine fish *Gillichthys mirabilis* (Family Gobiidae) and all of its parasites, a digenetic trematode was found on the gills, in the esophagus, and in the stomach. Since it is a new species and our efforts to determine its generic designation have disclosed some questions on the relationship between two genera, the species will be described before we continue our studies on the ecology of all the gill parasites and their host. In 1951 Manter and Van Cleave mentioned the presence of a larval hemiurid in this host collected at La Jolla, California. We assume that this worm is the same as the one being described, because we have found the parasite in *Gillichthys mirabilis* taken from Morro Bay and from Point Mugu, California. The descriptions given below are based on material collected at Goleta, California.

METHODS

The worms were fixed in warm Gilson's fluid and stored in either the same fluid or in 70 percent ethyl alcohol. They were studied while alive, or in the fixed, unstained condition, or after staining in either acetocarmine, paracarmine, alum haematoxylin, or celestine Blue B. They were mounted in polyvinyl alcohol, permount, or piccolyte. Longitudinal and cross sections were stained in alum haematoxylin. Approximately 600 worms were selected for this study.

The Genera *Brachadena* and *Hysterolecitha*

Generic diagnosis: *Brachadena.* Family Hemiuridae; subfamily Lecithasterinae. Body smooth, somewhat flattened, without ecsoma. Oral sucker subterminal; pharynx globular, contiguous with oral sucker; esophagus absent; ceca extend to posterior extremity. Acetabulum 2.5 to 3 times larger than oral sucker, situated in or at anterior body half. Genital pore ventral to pharynx or to cecal bifurcation. Testes symmetrical, lateral, immediately postacetabular. Seminal vesicle clavate, preacetabular. Pars prostatica longer than seminal vesicle, well developed, coiled, surrounded by large mass of prostate cells. Hermaphroditic duct muscular, enclosed in well developed pouch. Genital atrium absent. Ovary ovoid, unlobed, situated about midway between acetabulum and posterior end of body. Seminal receptacle just anterior to ovary. Vitellaria divided into 7 long digitiform lobes. Uterus extending posterior to ovary. Excretory crura uniting dorsal to pharynx or oral sucker.

Generic diagnosis: *Hysterolecitha.* Family Hemiuridae; subfamily Lecithasterinae. Body smooth, cylindrical, without ecsoma. Oral sucker subterminal; pharynx globular, contiguous with oral sucker; esophagus present or absent; ceca extending nearly to posterior extremity. Acetabulum 1.3 to 3 times larger than oral sucker, situated near anterior third of body. Genital pore ventral to pharynx or to cecal bifurcation. Testes diagonal to almost tandem, near to or some distance posterior to acetabulum. Seminal vesicle tubular to saccular, twisted, bent, or winding anterior to center of acetabulum. Pars prostatica either duct-like or vesicle-like; prostate gland variable in size. Hermaphroditic duct muscular, in some cases protrusible, enclosed in hermaphroditic pouch or not enclosed. Ovary ovoid, unlobed, in posterior half of body. Seminal receptacle lacking. Uterine seminal rectptacle present as portion of uterus posterior to vitelline complex. Vitellaria divided into 7 to 9 short clavate, elongate clavate, or digitiform lobes. Uterus extending posterior to ovary. Excretory crura uniting dosral to pharynx or oral sucker.

Both of these genera were described by Linton in 1910 from the intestines of fishes collected at Tortugas, Florida. Important morphological differences as reported by Linton, augmented by Manter (1947), and modified by us are shown in table I.

TABLE I. *Important morphological differences between* Brachadena *and* Hysterolecitha.

	Brachadena	*Hysterolecitha*
Testes	Transversely lateral	Diagonal, close together
Vitelline complex	7 elongated lobes	7 to 9 lobes, elongated or rounded
Seminal receptacle	Present	Absent
Body shape	Somewhat flattened	Elongate, cylindrical
Relative size of prostate gland	Large	Small to moderately large

In 1953 Yamaguti combined the genus

Received for publication January 18, 1961.
* This study was supported by a grant from the National Science Foundation.

Brachadena with the genus *Lecithophyllum* Odhner 1905. Bravo-Hollis (1956) and Margolis (1958), however, restored the separate generic status of *Brachadena*. This genus can be separated from *Lecithophyllum* by the centrally united vitellaria in the former. We have studied slides of *Brachadena pyriformis* obtained from Professor F. Sogandares-Bernal, and we have been unable to find a genital atrium in the worms. The absence of this atrium is an additional justification for separating the genus from *Lecithophyllum*. The related genus *Aponurus* is also without a genital atrium, but its vitellaria are formed into two separate groups.

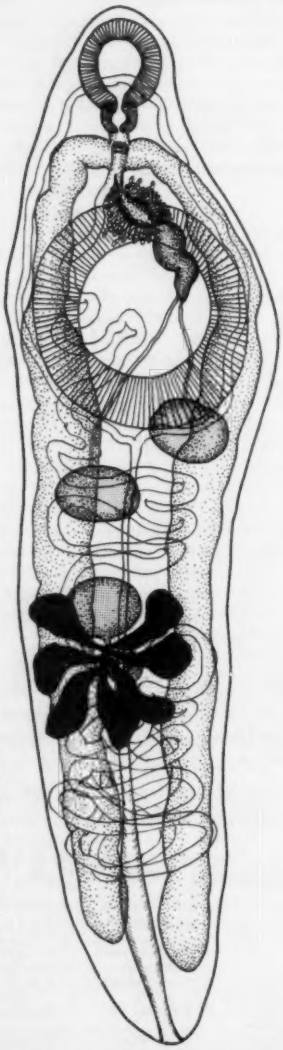


FIGURE 1. *Hysterolecitha trilocalis* n. sp. Composite drawing of mature adult. The uterine coils have been reduced to numbers below average conditions and eggs omitted in order not to obscure other organs. Ventral view.

A review of the descriptions of the eleven known species of *Hysterolecitha* shows that, except for the absence of a seminal receptacle, all diagnostic features vary in their ranges to include those described for *Brachadena*. Unfortunately, 8 of the 11 species descriptions of *Hysterolecitha* were based on only three or fewer worms, and often certain important details were not mentioned. Our experience has shown that individual variations are so pronounced that, in order to arrive at justifiable conclusions, large numbers of whole worms and sectioned material must be studied. It seems obvious, therefore, that the species of *Hysterolecitha* and *Brachadena* should be restudied with emphasis on variation of morphological details before final answers can be made to questions on this generic complex.

We have placed our species in the genus *Hysterolecitha* because (a) there is no seminal receptacle, (b) the testes are arranged diagonally, and (c) the general appearance and body proportions resemble the "typical" *Hysterolecitha*.

Hysterolecitha trilocalis n. sp.

Specific diagnosis: All measurements are in millimeters and are of fixed specimens, based on 25 measurements in each case. Averages are in parenthesis.

Hysterolecitha: small and cylindrical; slightly tapered at both extremities; reddish in color in life. Body 1.55 to 4.22 (3.05) long by 0.40 to 1.11 (0.81) wide at the level of the acetabulum. Mouth subterminal, surrounded by three pairs of papillae; no preoral lip. Oral sucker round to oval, 0.16 to 0.48 (0.28) long by 0.18 to 0.36 (0.27) wide. Acetabulum 0.32 to 0.78 (0.59) long by 0.32 to 0.76 (0.56) wide. Average distance from center of acetabulum to anterior end equaling 26.3 percent total body length. Sucker ratio 1:1.45 to 2.62 (1: 1.97). Pharynx 0.07 to 0.15 (0.10) long by 0.06 to 0.11 (0.09) wide, contiguous with oral sucker. Prepharynx absent. Esophagus short, narrow, dorsally perpendicular to pharynx on dorso-ventral plane. Ceca variable in width, extending nearly to posterior extremity. Genital pore median, ventral to pharynx or to cecal bifurcation. Seminal vesicle long, saccular, having 2 or 3 distinct bends; sometimes extending posterior to anterior margin of

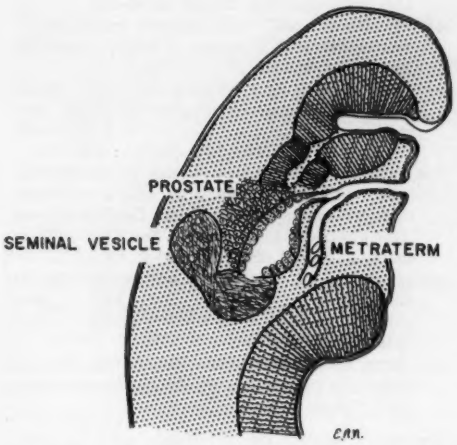


FIGURE 2. Longitudinal section of anterior end of *Hysterolecitha trilocalis* to show details of seminal vesicle, prostate, metraterm, and genital pore. Prostate gland cell mass is shown on one side of the prostate.

acetabulum. Seminal vesicle, constricted at opening, opens into wide, pyriform prostatic vesicle, lined with columnar glandular cells. Prostatic vesicle one-half as long as seminal vesicle and surrounded by large cluster of glandular cells. Hermaphroditic duct muscular. Vasa efferentia not united to form vas deferens, but opening directly into seminal vesicle. Testes 2, diagonal, ovoid, equal or subequal in size; anterior testis 0.14 to 0.40 by 0.10 to 0.28 (0.25 by 0.18), posterior testis 0.14 to 0.37 by 0.11 to 0.28 (0.24 by 0.19). Average distance between posterior margin of acetabulum and most anterior testis equaling 1.3 percent of total body length.

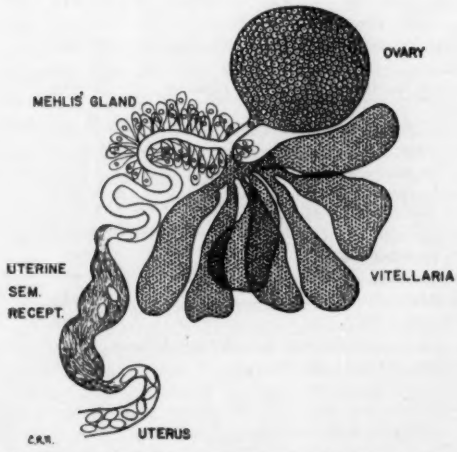


FIGURE 3. Details of female reproductive organs of *Hysterolecitha trilocalis*.

Ovary oval, 0.12 to 0.32 by 0.08 to 0.30 (0.22 by 0.19). Average distance from ovary to anterior end of body equaling 56.5 percent of total body length. Average distance between ovary and most posterior testis equaling 6.2 percent of total body length. Vitellaria just posterior to ovary; composed of 7 digitiform or long clavate lobes united centrally. Uterine coils extending posterior to vitellaria, but not posterior to cecal ends, then ascending in thick loops to posterior margin of acetabulum, where uterus narrows and extends, with little or no coiling, directly to genital complex. Space between acetabulum and ovary usually densely packed with uterine material, sometimes obscuring testes. Uterine seminal receptacle present as enlarged portion of uterus situated just posterior to vitellaria. Excretory pore terminal; excretory bladder tubular, slightly inflated, constricting into slender tube, latter bifurcating posterior to acetabulum, arms continuing anteriorly and uniting dorsal to pharynx or oral sucker. Eggs 0.027 to 0.029 long by 0.012 to 0.016 wide.

This species is named for the three sites of infection in the type host.

Type species: Hysterolecitha rosea Linton, 1910.

Host: Gillichthys mirabilis Cooper: the "mudsucker" or "long-jawed goby."

Location: Gills, esophagus, stomach.

Locality: Goleta, Santa Barbara County, California.

Holotype and paratype, U.S.N.M. Helm. Coll. No. 39081.

The following provisional key to the species of the genus *Hysterolecitha* Linton 1910, is based on descriptions in the literature. *H. blepsiae* Layman 1930, has been omitted because of insufficient specific diagnosis. This key is an enlargement of one proposed by Srivastava, 1937.

Ovary and vitellaria close behind middle of body . 1
Ovary and vitellaria in last third of body 3
1 Esophagus present ..*H. trilocalis* King and Noble.
Esophagus absent 2
2 Genital pore at pharynx..*H. elongata* Manter, 1931.
Genital pore posterior to cecal bifurcation. *H. acanthuri* Annereaux, 1947.
3 Esophagus present 4
Esophagus absent 9
4 Eggs filamented ..*H. tinkeri* Manter and Pritchard, 1960.
Eggs not filamented 5
5 Vitellaria compact mass, short lobes 6
Vitellaria elnogate, digitiform lobes 7
6 Sucker ratio 1 : 3 ; thin-walled sinus sac..*H. rosea* Linton, 1910.
Sucker ratio 1 : 2 ; thick-walled sinus sac..*H. crassivesiculata* Bravo-Hollis, 1956.
7 Testes much smaller than ovary..*H. microrchis* Yamaguti, 1934.
Testes equal to or subequal to ovary 8
8 Hermaphroditic pouch globular, large; vitellaria of 7 elongate lobes..... *H. xesuri* Yamaguti, 1938.
Hermaphroditic pouch pyriform, small ; vitellaria of 7 or 8 clavate lobes *H. nahaensis* Yamaguti, 1942.
9 Vitellaria of 8 elongate lobes..*H. lintoni* Srivastava, 1937
Vitellaria of 7 digitiform lobes..*H. teuthis* Nagaty, 1956.

SUMMARY

Hysterolecitha trilocalis n.sp. (Trematoda: Hemiuridae) is described from the gills, esophagus, and stomach of the goby *Gillichthys mirabilis*. The genus *Brachadena* is compared with *Hysterolecitha*, and diagnostic characteristics of each genus are presented. A key to species of *Hysterolecitha* is added. Material for the study was collected at Goleta, California.

LITERATURE CITED

BRAVO-HOLLIS, M. 1956 Trematodos de peces marinos de aguas Mexicanas. XI. Estudio de 17 digeneos de las costas del Pacifico, incluyendo, seis especies nuevas y un genero nuevo. Anales Inst. Biol. Mex. **27**: 245–277.

LINTON, E. 1910 Helminth fauna of the Dry Tortugas. II. Trematodes. Papers Tortugas Lab. Carnegie Inst. Wash: **4**: 11–98.

MANTER, H. W. 1947 The digenetic trematodes of marine fishes of Tortugas, Florida. Am. Midl. Nat. **38**: 396–411.

——— AND VAN CLEAVE, H. J. 1951 Some digenetic trematodes, including eight new species, from marine fishes of La Jolla, Calif. Proc. U.S. Natl. Mus. **101**: (3279): 315–340.

MARGOLIS, L. 1958 A new species of *Lecithophyllum* from north Pacific fishes with a consideration of taxonomy of the genera *Lecithophyllum*, *Aponurus*, and *Brachadena* (Trematoda; Hemiuridae). Can. J. Zool. **36**: 983–904.

SRIVASTAVA, H. D. 1937 New hemiurids from Indian marine food fishes. Part VII. A new parasite of the genus *Hysterolecitha* Linton, 1910. Ind. J. Vet. Sci. **9**: 73–76.

YAMAGUTI, S. 1953 Systema Helminthum. Part I. Digenetic Trematodes of Fishes. Maruzen Co., Ltd., Tokyo.

(*Continued from page 365*)

Jan. 29–Feb. 9	Laboratory methods in the diagnosis of tuberculosis (855). Closing date: December 18, 1961.	2 wks
Jan. 29–Feb. 9	Serologic methods in microbiology (941). Closing Date: December 18, 1961.	2 wks
Feb. 12–23	Laboratory methods in the study of pulmonary mycoses (817). Closing date: January 2, 1962.	2 wks
Feb. 12–23	Fundamentals of virology (819). Closing date: January 2, 1962.	2 wks
Feb. 26–Mar. 16	Laboratory methods in medical bacteriology (838). Closing date: January 15, 1962.	3 wks
Mar. 5–9	Laboratory diagnostic methods in veterinary mycology (940). Closing date: January 22, 1962.	1 wk
Mar. 12–30	Laboratory methods in the diagnosis of viral and rickettsial diseases (820). Closing date: January 29, 1962.	3 wks
Mar. 19–23	Special problems in medical bacteriology (839). Closing date: February 5, 1962.	1 wk
Mar. 26–Apr. 6	Laboratory methods in enteric bacteriology (850). Closing date: February 12, 1962.	2 wks
Apr. 9–13	Laboratory methods in the diagnosis of rabies (826). Closing date: February 26, 1962.	1 wk
Courses given by special arrangement	Laboratory methods in the diagnosis of malaria (805).	1 wk
	Special training in virus techniques (821).	2–4 wks
	Typing of *Corynebacterium diphtheriae* (842).	1 wk
	Special problems in enteric bacteriology (851)	2 wks
	Phage typing of *Salmonella typhosa* (852).	1 wk
	Laboratory methods in the diagnosis of leptospirosis (853).	1–4 wks
	Serologic differentiation of streptococci (854).	2 wks
	Special problems in microbiology (942).	1–2 wks

Information and application forms may be obtained from the Laboratory Branch, Communicable Disease Center, U.S. Public Health Service, Atlanta 22, Georgia.

DESCRIPTION, LIFE HISTORY, AND DEVELOPMENTAL PATTERN OF *GLYPTHELMINS PENNSYLVANIENSIS* N. SP. (TREMATODA: BRACHYCOELIIDAE), NEW PARASITE OF FROGS*

THOMAS C. CHENG
Lafayette College, Easton, Pennsylvania

The trematode genus *Glypthelmins* was erected by Stafford (1905) to include *Distomum quietum* Stafford, 1900, an intestinal parasite of *Rana catesbeiana, R. virescens,* and *Hyla pickeringii* in Canada. Since that time numerous species have been assigned to this genus by various workers; however, this author (Cheng, 1959) reviewed the known species and transferred all the species to the genus *Margeana* Cort, 1913, except the type species, *G. quieta,* and two others, *G. subtropica* Harwood, 1932, and *G. festina* Cordero, 1944. The reason given for the transfer of the remaining species to *Margeana* was that only the three above-mentioned species possess peripharyngeal glands which is one of the diagonstic characters given by Stafford in establishing *Glypthelmins.* It was felt that the presence of these unique glands, which empty into the muscular pharynix, is of sufficient consistency and prominence to be employed as a generic character. Taxonomic keys to the species of *Glypthelmins* and *Margeana* have been given by Cheng (loc. cit.).

As the result of a continuous survey of the parasitic fauna of eastern Pennsylvania, over 200 specimens of an intestinal trematode were obtained from the small intestine of the spring peeper, *Hyla crucifer* Wied, captured at Lake Warren, State Game Reserve No. 56, in Bucks County, Pennsylvania. A total of 40 frogs were examined to date from this locale. The number of flukes found in each host varied from 2 to 12. Critical examination of these trematodes revealed sufficient consistent differences to warrant their description as representing a new species, *Glypthelmins pennsylvaniensis.*

Rankin (1944) and Leigh (1937, 1946) independently reported the life history of *Glypthelminus quieta.* These workers stated that during the developmental cycle of this species the snail *Physa gyrina* serves as the only intermediate host. Within the molluse, the parasite undergoes two sporocyst generations. The escaping fin-tailed xiphidiocercariae, which belong to the Ornatae group of Lühe (1909), were found to be identical with *Cercaria mesotyphla* which had been described earlier by Miller (1935). These cercariae penetrate and encyst as metacercariae in the skin of *Rana pipiens;* when the sloughed epithelia are ingested by the frog, the life cycle of the species is completed.

The established life history pattern in the genus *Glypthelmins* facilitated the determination of the developmental pattern of *G. pennsylvaniensis.* A survey of the molluses in Lake Warren revealed that of the five species found, *Helisoma trivolvis* (Say), taken from the eastern shore of the lake in the same area where the infected frogs were captured, emitted a xiphidiocercaria which is similar to that of *G. quieta.* It was strongly suspected that the author's cercaria was the larva of the new trematode, and subsequent experiments were carried out to test this hypothesis.

The earlier reports of the encystment of the metacercaria of *G. quieta* in the skin of the definitive host, however, puzzled this author, since in *Brachycoelium obesum* Nicoll, 1914, and *Mesocoelium lanceatum* Goto and Ozaki, 1929 (= *M. brevicaecum* Ochi, 1930), the two other members of the Brachycoeliidae, the life histories of which are known, Cheng (1960a) and Ochi (1930) reported that the cercariae lose their tails but remain unencysted in the mollusean hosts, and that when the snails are ingested by the definitive hosts, the cycles are

Received for publication November 28, 1960.
* A contribution from the Jenks Biological Laboratory, Lafayette College, made possible (in part) through Grant E-3443 from the Division of Allergy and Infectious Diseases, National Institutes of Health. The author is grateful to Dr. Joseph P. E. Morrison, Curator of Mollusks, The Smithsonian Institution, U.S. National Museum, Washington, D.C., for identifying the snail host.

completed. It was suspected that perhaps the members of the family Brachycoeliidae are capable of both avenues of development. For this reason the infected snails were carefully examined for non-encysted metacercariae, but none were found. A concentrated effort was also made to collect various arthropods in the immediate area in order to determine whether a second intermediate host could be utilized in the life cycle of *Glypthelmins pennsylvaniensis* in place of the epithelia of the definitive host.

During the course of this study, the complete life history of *G. pennsylvaniensis* was successfully demonstrated both in nature and under experimental conditions in the laboratory. Certain distinct differences in the morphology and host specificity between *G. quieta* and the new species were noted; these further validate the recognition of *G. pennsylvaniensis* as being new. The general pattern of the life histories of the two species, i.e., the requirement of only one intermediate host, was established, thus strongly suggesting the obvious close relationship between the two species and further strengthening the author's earlier contention (Cheng, 1958, 1959, 1960a, b) that the genus *Glypthelmins* is closely related to *Brachycoelium* Looss, 1899, and *Mesocoelium* Odhner, 1911, since, as mentioned, in the life cycles of both *B. obesum* and *M. lanceatum* only one intermediate host is required.

MATERIALS AND METHODS

The sexually mature worms used in the description of the new species were all obtained from naturally infected *Hyla crucifer* captured at Lake Warren since adults recovered from experimentally infected frogs were not sexually mature. The specimens were fixed in Carnoy's (6 : 1 : 1) under slight pressure, stored in 70 percent alcohol, and later stained with Harris' alum haematoxylin.

Uninfected specimens of the gastropod host, *Helisoma trivolvis*, used as experimental hosts were obtained from another nearby lake where a continuous survey over the months of March through September 1960 revealed no helminth infections. The apparently non-parasitized condition of these snails was verified by crushing and examining 10 to 15 specimens from the same location at each collection during the seven month period. A total of 15 collections were made. Uninfected specimens of *Hyla crucifer* used in experimental infections were raised in the laboratory from eggs.

To determine the susceptibility of *Helisoma trivolvis* experimentally, uninfected snails were fed eggs of the worm isolated from the feces of naturally infected hosts by placing approximately 100 eggs on a piece of lettuce. Such pieces of lettuce were then placed in finger bowls in which uninfected snails had been singularly isolated in distilled water. After it was determined that some of the snails had become infected, two series, 30 per series, of cercariae-elaborating snails were set up; one series of naturally infected and the other of laboratory infected snails. Two uninfected frogs were placed in each finger bowl. The frogs were left in the bowls for a period of 84 hours during which time they were not fed. After the 84-hour exposure to cercariae escaping from the snails, the frogs were autopsied and examined. One to three young adults were recovered from the small intestine of all the frogs. These were fixed in Carnoy's and stained with Harris' alum haematoxylin. In addition to examining the intestine of these experimentally infected frogs, pieces of their skin were removed, fixed, sectioned, and stained with Mallory's triple connective tissue stain.

Ten specimens of *Helisoma trivolvis* were removed from their shells 10 to 30 days after they were experimentally infected and dissected apart in order to study the larval generations of the trematode within their tissues. Since Leigh (1946), Cort and Olivier (1943), and Cort et al (1954) had reported that the mother sporocysts of some members of the Plagiorchioidea are not found in or adjacent to the mollusc's hepatopancreas, but are attached to the snail's alimentary tract, great care was taken to examine the intestine of these snails for mother sporocysts, which were found attached along the length of the intestine. Four additional infected snails were removed from their shells and fixed in Carnoy's, sectioned at 10 microns, and the sections were stained with Mallory's triple and Heidenhain's iron haematoxylin in order to study the histology and cytology of the sporocysts and cercariae.

In another series of experiments, eggs were fed to uninfected snails in the manner described above; however, the snails were examined at 10-, 20-, 30- and 60-minute intervals, and miracidia were looked for in their alimentary tracts. Active miracidia were observed in these snails at the 20- and 30-minute observations. This finding suggests that the developed miracidium of *G. pennsylvaniensis* does not become active, nor do they hatch until the eggs come in contact with the digestive juices of the molluscan host.

The miracidia, mother and daughter sporocysts, and cercariae were all studied in the living state with both light and phase-contrast microscopes. The encysted metacercariae were studied both in the living state with phase and as stained specimens which were previously fixed with Carnoy's.

As mentioned earlier, various arthropods were collected from the area where naturally infected *Helisoma trivolvis* and *Hyla crucifer* were found. These arthropods included mayfly and dragonfly nymphs, various beetles, and decapods. These were exposed to naturally infected snails; however, when they were subsequently examined at 48-, 72- and 94-hour intervals, none were found to harbor meta-

cercariae. Hence, they were considered not suitable as second intermediate hosts; in fact, no second intermediate host was found to be necessary. Since these arthropods were unimportant in the completion of the life cycle of the trematode, they were not identified to species.

The morphology and locations of the stages in the life cycle of *Glypthelmins pennsylvaniensis* are given below; the cytological observations on this species will be reported elsewhere.

All measurements were made with a calibrated eye-piece micrometer and given in millimeters.

STAGES IN THE LIFE CYCLE

The Adult (Plate I, figs. 1, 2)

The following description of the sexually mature adult of *Glypthelmins pennsylvaniensis* n. sp. is based on 10 specimens randomly selected from 10 naturally infected *Hyla crucifer*. All measurements were made with an eyepiece micrometer and given in millimeters.

Description: Brachycoeliidae. Elongate distome, 1.314 to 2.88 (average 1.841) long, 0.45 to 0.9 (average 0.641) wide; cuticle spinous, spines more dense anteriorly; anterior sucker subterminal, 0.132 to 0.264 (average 0.193) long by 0.145 to 0.264 (average 0.189) wide; acetabulum in anterior half of body, 0.125 to 0.198 (average 0.15) long by 0.132 to 0.231 (average 0.168) wide; inconspicuous prepharynx present, pharynx 0.099 to 0.139 (average 0.115) in transverse diameter, esophagus 0.066 to 0.165 (average 0.102) long, bifurcating approximately midway between posterior margin of pharynx and anterior margin of acetabulum, intestinal ceca averaging 0.462 long, reaching into posterior one-fourth of body length, lined with single layer of cells; 5 to 6 prominent peripharyngeal glands present on each side of and emptying into pharynx, each unicellular gland measuring 0.029 to 0.053 (average 0.045) long by 0.026 to 0.042 (average 0.033) wide; testes obliquely situated in posterior half of body, left testis 0.092 to 0.298 (average 0.181) by 0.086 to 0.168 (average 0.126), right testis 0.086 to 0.224 (average 0.17) by 0.072 to 0.165 (average 0.135), left testis more anteriorly situated than its mate; cirrus pouch, 0.075 to 0.165 long (average 0.118), 0.042 to 0.077 (average 0.061) wide, partially overlapping acetabulum and opening to exterior through common genital pore on midline immediately anterior to acetabulum; cirrus pouch containing seminal vesicle (average 0.091 by 0.047) and coiled cirrus (average 0.118 long and 0.061 wide); ovary, 0.059 to 0.152 (average 0.092) by 0.046 to 0.099 (average 0.073), anterior to testes, immediately posterior to and partially overlapping acetabulum; oötype, 0.033 in diameter, immediately posterior to ovary (plate I, fig. 3); left and right vitelline ducts entering oötype; seminal receptacle, averaging 0.072 in diameter, posterior to and overlapping ovary; vitellaria of distinct follicles lateral to intestinal ceca, extending from level of midlength of esophagus to level of posterior testis; excretory pore terminal; excretory vesicle tubular, extending anteriorly beyond level of testes. Eggs operculate, 0.03 to 0.038 by 0.015 to 0.021 (average 0.034 by 0.019) situated in coiled ascending and descending limbs of uterus.

Type host: Hyla crucifer Wied.

Type locality: Lake Warren, State Game Reserve No. 56, Bucks County, Pennsylvania.

Type and paratype specimens: USNM Helm. Coll. No. 59515.

Glypthelmins pennsylvaniensis can be distinguished from *G. quieta* and *G. subtropica* by the configuration of its vitellaria. In *G. pennsylvaniensis* the vitelline follicles are distinctly extracecal in position, in *G. quieta* they are extracecal and cecal, and in *G. subtropica* the minute follicles are extracecal, intercecal and cecal. *G. pennsylvaniensis* can be further distinguished from *G. quieta* by the length of its intestinal ceca which do not extend to the extreme posterior end of the body as is the case in *G. quieta*, and by the position of its gonads since in the new species the ovary is located posterior to the acetabulum and the obliquely arranged testes are located in the posterior half of the body while in *G. quieta* the ovary is anterior to the acetabulum and the testes are more anteriorly situated and are placed side by side. *G. pennsylvaniensis* can be further distinguished from *G. subtropica* by the position of its ovary since in the latter species the ovary is on the same level as and lateral to the acetabulum.

Glypthelmins pennsylvaniensis most closely resembles *G. festina;* however, it can be distinguished from the latter by the size and position of its gonads. The spherical ovary of the new species, measuring 0.059 to 0.152 mm by 0.048 to 0.099 mm, is approximately half the size of the ovoid ovary of *G. festina* which measures 0.21 to 0.275 mm by 0.145 to 0.23 mm, and does not overlap the right intestinal cecum as it does in *G. festina*. Again, the testes of *G. festina* are approximately 2.5 to 3 times larger than those of *G. pennsylvaniensis* and not only overlap each other, but overlap the intestinal ceca; the testes of the new species do not overlap the ceca or each other.

The Egg (Plate I, fig. 4)

The eggs of *Glypthelmins pennsylvaniensis* are distinctly operculate. Those seen in the descending limb of the convoluted uterus include developing embryos and yolk globules, those seen at the terminal end of the uterus include

immotile but fairly well developed miracidia.

The Miracidium (Plate I, fig. 7)

The miracidium of *G. pennsylvaniensis* is formed within the egg-shell before leaving the parent; however, it remains immotile until the egg comes in contact with the snail host's digestive juices at which time it actively escapes from the shell. The following description is based on five living specimens removed from the intestine of experimentally infected *Helisoma trivolvis* and studied under phase.

Description: Body ovoid, 0.025 by 0.015, covered with cilia of uniform length; distinct nonciliated apical papilla present; 2 to 4 germ balls visible towards posterior end of body; actively motile.

Miracidia were not observed in the process of penetrating the epithelial lining of the snail's intestine; however, since the mother sporocysts were found attached to the outside of the intestine it appears reasonable to postulate that the miracidia do penetrate through the intestinal wall.

The Mother Sporocyst (Plate I, figs. 5, 6; plate II, figs. 8, 9)

The mother or first-generation sporocysts of *Glypthelmins pennsylvaniensis* were found attached along the intestine of the snail host by an extremely thin transparent ligament-like sac the ends of which are drawn out. A diligent search along the entire lengths of the intestine of 10 experimentally infected snails revealed mother sporocysts in relatively large numbers, approximately 15 to 35 per snail, in all stages of development.

Description: Youngest mother sporocysts found (plate I, fig. 5): spindle-shaped, averaging 0.025 by 0.009; brood chamber including 4 typical germinal cells; sporocyst wall smooth and transparent. *Second stage mother sporocysts* (plate I, fig. 6): measuring 0.348 by 0.139; similar to youngest specimens but larger; brood chamber including somatic cells in addition to germinal cells; somatic cells distinguishable from germinal cells by their smaller size, less prominent nucleus, and lack of spoke-like radiations arising from nucleus when seen in sections stained with Heidenhain's iron haematoxylin. *Third stage mother sporocysts* (plate 2, fig. 8): averaging 0.425 by 0.17 (most specimens smaller); sporocyst wall comparatively thick with surficial protrusions; brood chamber enclosing cell aggregates rather than individual cells suggesting that the original cells had undergone division. *Oldest mother sporocysts* (plate 2, fig. 9): densely opaque; 0.609 by 0.313; brood chamber filled with numerous cell aggregates, each of which will give rise to a young daughter sporocyst (plate 2, fig. 10).

All the cell aggregates or germ balls within a mother sporocyst develop at approximately the same rate. When these develop into young daughter sporocysts the latter rupture out of the mother sporocyst and destroy the latter in the process. Young daughter sporocysts become free in the body cavity of the snail and apparently migrate to the hepatopancreas since large numbers of daughter sporocysts were found in that organ.

The Daughter Sporocyst (Plate II, figs. 10, 11, 14)

The young daughter or second-generation sporocysts escaping from the mother sporocyst are sausage-shaped, measuring 0.058 long by 0.012 wide. Within each individual can be seen a reproductive cell which possesses the characteristically darker and larger nucleus. The brood chamber is inconspicuous since it is filled with loosely packed cells. Upon reaching the snail's hepatopancreas, the daughter sporocysts begin to grow and continue their differentiation. A single hepatopancreas may contain as many as 400 to 500 daughter sporocysts.

The following description of the mature daughter sporocyst is based on 15 specimens removed from experimentally infected snails.

Description: Body elongate and transparent, 0.35 to 0.48 (average 0.42) long, 0.05 to 0.12 (average 0.09); birthpore present at anterior end (plate 2, fig. 14); sporocyst wall relatively thick; brood chamber including germ balls and cercariae in all stages of morphogenesis, and a germinal mass composed of 10 to 15 germinal cells; each sporocyst enclosing 8 to 17 cercariae.

Fully developed cercariae escape through the birthpore of the daughter sporocyst, actively migrate from the hepatopancreas, and escape from the snail.

The Cercaria (Plate II, fig. 12)

The cercariae of *Glypthelmins pennsylvaniensis* are active swimmers. When observed in a finger bowl, they flicker about in the water by flexing both the tail and the body proper. Periodically, they cease swimming and settle to the bottom of the bowl where some actively inch about by utilizing their powerful suckers.

The following description of the cercaria is based on 15 specimens.

Description: Elongate distomate xiphidiocer-

caria; body proper 0.088 to 0.185 (average 0.109) long, 0.031 to 0.079 (average 0.061) wide; cuticle spinous; tail 0.048 to 0.079 (average 0.052) long, 0.005 to 0.019 (average 0.009) wide; anterior sucker 0.023 to 0.03 (average 0.025) by 0.022 to 0.03 (average 0.025); stylet (plate II, fig. 13) 0.006 to 0.011 (average 0.008) long, 0.002 to 0.004 (average 0.003) wide at the broadest point; prepharynx visible; muscular pharynx 0.008 to 0.013 (average 0.01) in diameter, esophagus 0.011 to 0.024 (average 0.017) long, bifurcating near midlength of body giving rise to intestinal ceca which terminate at level of acetabulum; acetabulum, 0.02 to 0.026 (average 0.023) by 0.02 to 0.028 (average 0.023), in posterior half of body; 12 conspicuous penetration glands present, averaging 0.015 by 0.011 each, 6 on each side of and anterolaterad to acetabulum; primordia of peripharyngeal glands visible in some specimens located on each side of pharynx; genital primordium on midline posterior to and partially overlapping acetabulum; excretory vesicle large, 0.035 in transverse diameter, lined with mesodermal cells, actively pulsating; flame-cell formula 2[(3+3+3)+(3+3+3)].

The Metacercaria (Plate II, fig. 15)

A careful search of the body surface of naturally infected *Hyla crucifer* did not reveal any metacercariae encysted in their skin, nor did examinations of the skin sections of experimentally infected frogs. A concentrated search over the entire body surface of one experimentally infected frog, however, revealed three slight bulges which, when dissected apart, were found to contain an encysted metacercaria each. It is apparent that in experimental infections the number of encysted metacercariae in the definitive host's skin are not plentiful; in the case of this series of studies, the majority of the metacercariae had been sloughed off and ingested since, as mentioned, young adults were recovered from experimental hosts. It was not surprising that no metacercariae were found in naturally infected frogs, since they undoubtedly had long been sloughed off; reinfections were not possible because collected frogs were maintained for at least 7 days, usually longer, in the laboratory without coming in contact with infected snails before they were examined.

The three encysted metacercariae were removed from their host and studied, first with phase, later fixed and stained. There is only one rather thin noncellular opaque cyst wall in which the worm is coiled ventrally. The entire cyst measures 0.315 to 0.385 in diameter. Rudimentary testes can be seen obliquely arranged in the posterior half of the body.

The Young Adult (Plate I, fig. 1)

The young adults recovered from the small intestine of experimentally infected frogs are consistently smaller than the sexually mature adults obtained from naturally infected hosts. The young adults were fixed in Carnoy's, stained and mounted for study. All the young adults were sexually immature since no eggs were found in the uteri. Furthermore, the vitellaria are composed of smaller follicles and the peripharyngeal glands are smaller.

DISCUSSION

By comparing the morphology of the various stages in the life history of *Glypthelmins pennsylvaniensis* with that of *G. quieta* as given by Miller (1930), Rankin (1944) and Leigh (1946), it is apparent that the two species are distinct, since several striking differences exist, not only in the sexually mature adults, but in the larval forms as well. The most striking differences between the two species lie in the morphology of the cercariae. The cercariae of *G. quieta* possesses a ventral fin on its tail while that of *G. pennsylvaniensis* does not. Miller (1935) and Leigh (1946) both reported the presence of 12 penetration glands in the cercaria of *G. quieta;* however, these are smaller and more anteriorly located than the 12 penetration glands of *G. pennsylvaniensis* (see Leigh, 1946: 482). No miracidial penetration glands were observed in the cercaria of *G. pennsylvaniensis* as was pictured by Leigh (1946) in the cercaria of *G. quieta;* however, this is presumably due to the degree of maturation of the specimens studied since such glands disappear when cercariae become fully developed. The cecal bifurcation in the cercaria of *G. quieta* is considerably more anterior in position than that of *G. pennsylvaniensis* and the stylet of the cercaria of *G. quieta* is definitely different from that of *G. pennsylvaniensis.* All three investigators of *G. quieta* found that *Physa gyrina* serves as the intermediate host for this species. Leigh (1946) reported that *Physa halei* can be utilized as the intermediate host. In the case of *G. pennsylvaniensis,* only *Helisoma trivolvis* of five species of snails, including *Physa* sp., found in the immediate area is suitable for the intermediate host for this species. The comparison of sexually mature adults of the two species has been given above.

The life cycles of the two species parallel each other. It is interesting to note that Leigh (1946: 481) showed an optical section of a 36-day-old mother sporocyst of *G. quieta* which possesses bulging walls much in the same fashion as the older mother sporocysts of *G. pennsylvaniensis*.

Concerning the development and morphology of the mother sporocyst among the Plagiorchioidea, Cort and Olivier (1943) and Cort and Ameel (1944) reported that in the life history of *Plagiorchis muris* Tanabe, 1922, the mother sporocysts form invaginating septa which divide the brood chamber into compartments within which individual developing daughter sporocysts are found; the septa eventually envelop the daughter sporocysts as the outermost layer, the paletot. Mattes (1936) reported that in *Dicrocoelium dendriticum* (Rud., 1818) Looss, 1845, the mother sporocysts are in the form of irregular masses located in the interlobal spaces of the snail host's hepatopancreas. Cort et al (1952) reported that in *Lechriorchis primus* Stafford, 1905, the mother sporocysts increase in size with the rapid multiplication of the enclosed germinal cells and eventually branch, with the branches extending into the snail's hepatopancreas. Again, Cort and Ameel (1944) reported that the mother sporocysts of *Macroderoides typicus* (Winfield, 1929) Van Cleave and Mueller, 1932, appear as tubules radiating from a common center. These reports, together with the herein reported superficial lobing of the mother sporocysts of *G. quieta* and *G. pennsylvaniensis*, suggest that in the Plagiorchioidea there is at least a tendency to form partitions during the maturation of the first generation sporocysts.

Cort et al (1954) have reviewed the life history and developmental pattern in the superfamily Plagiorchioidea. It is apparent that in most species in which the life cycles are known, the mother sporocysts are found outside of the gastropod hepatopancreas and the daughter sporocysts either reach that organ by actively migrating to it as in *G. pennsylvaniensis*, or the mother sporocysts penetrate the interlobular septa of that organ and the daughters are ruptured into it as in *Lechriorchis primus*. The consistency of the general pattern of the mother sporocysts being outside of the hepatopancreas led this author to reexamine his slides of cross-sections of the hepatopancreas of *Zonatoides ligerus* infected with the larvae of *Brachycoelium obesum* Nicoll, 1914, the life cycle of which species has been reported previously (Cheng, 1960a). The reevaluation of these slides resulted in the necessity of making a correction to earlier observations. The mother sporocysts of *B. obesum* are not embedded in the hepatopancreas proper, but lie in the interlobal septa of that organ. This observation indicates that *B. obesum* (Brachycoeliidae: Plagiorchioidea) does conform to the developmental pattern as found among the members of the Plagiorchioidea.

It is now established that two species of the genus *Glypthelmins* portray the same life history pattern, i.e., utilize only one intermediate host in which they undergo two sporocyst generations, and the escaping xiphidiocercariae encyst in the skin of their amphibian hosts. It is also apparent that in the family Brachycoeliidae Johnston, 1912, the metacercariae of some species do encyst as in *G. quieta* and *G. pennsylvaniensis*, while in others, as in *Brachycoelium obesum* and *Mesocoelium lanceatum*, the metacercariae do not encyst, but remain in the gastropod hosts and eventually reach the definitive host only if infected snails are ingested.

The function of the peripharyngeal glands found in adults of the genus *Glypthelmins* remains hazy, although it is suspected that they serve in digestive and lubricative roles. The appearance of the primordia of these glands during the cercarial stage, distinct from the cercarial penetration glands and the "miracidial penetration glands" as noted by Leigh (1946), and the completion of their development in the adult definitely indicate that they are adult structures peculiar to members of the genus and, hence, must be considered a reliable character in distinguishing between the adult members of the closely related genera *Glypthelmins* and *Margeana*.

LITERATURE CITED

CHENG, T. C. 1958 Studies on the trematode family Dicrocoeliidae I. The Genera *Brachycoelium* (Dujardin, 1845) and *Leptophallus* Lühe, 1909, (Brachycoeliinae). Am. Midl. Nat. 59: 67–81.

———— 1959 Studies on the trematode family Brachycoeliidae II Revision of the genera *Glypthelmins* (Stafford, 1900) Stafford, 1905, and *Margeana* Cort, 1919; and the description of *Reynoldstrema* n. gen. (Glypthelminae n. subfam.). Am. Midl. Nat. 61: 68–88.

———— 1960a The life history of *Brachycoelium obesum* Nicoll, 1914, with a discussion of

the systematic status of the trematode family Brachycoeliidae Johnston, 1912. J. Parasit. **46**: 464–474.

———— 1960b Studies on the trematode family Brachycoeliidae. IV. A revision of the Genus *Mesocoelium* Odhner, 1911, and the Status of *Pintnaria* Poche, 1907. Am. Midl. Nat. **63**: 439–469.

CORT, W. W. and AMEEL, D. J. 1944 Further studies on the development of the sporocyst stages of plagiorchiid trematodes. J. Parasit. **30**: 37–50.

———— and OLIVIER, L. 1943 The development of the larval stages of *Plagiorchis muris* Tanabe, 1922, in the first intermediate host. J. Parasit. **29**: 81–99.

————, AMEEL, D. J. and VAN DER WOUDE, A. 1952 Development of the mother and daughter sporocysts of a snake plagiorchioid, *Lechriorchis primus* (Trematoda: Reniferidae). J. Parasit. **38**: 187–202.

CORT, W. W., AMEEL, D. J. and VAN DER WOUDE, A. 1954 Germinal development in the sporocysts and rediae of the digenetic trematodes. Exper. Parasit. **3**: 185–225.

LEIGH, W. H. 1937 The life cycle of a trematode of frogs. Science **86**: 423.

———— 1946 Experimental studies on the life cycle of *Glypthelmins quieta* (Stafford, 1900), a trematode of frogs. Am. Midl. Nat. **35**: 460–483.

LÜHE, M. 1909 Parasitische Plattwürmer. I. Trematodes. Die Süsswasserfauna Deutschlunds **17**: 1–217.

MATTES, O. 1936 Der Entwicklungsgang des Lanzettegel, *Dicrocoelium dendriticum*. Z. Parasitenk. **8**: 371–440.

MILLER, E. L. 1930 Studies on *Glypthelmins quieta* Stafford. J. Parasit. **16**: 237–243.

———— 1935 Studies on North American cercariae (Abstract). J. Parasit. **21**: 244–254.

OCHI, S. 1930 Ueber de Entwickelungeschiste von *"Mesocoelium brevicaecum"* n. sp. Okag. Ygakk. Zasshi Jg. **42**: 311–399.

RANKIN, J. S., JR. 1944 A review of the trematode genus *Glypthelmins* Stafford, 1905, with an account of the life cycle of *G. quieta* (Stafford, 1900) Stafford, 1905. Tr. Am. Micr. Soc. **63**: 30–43.

STAFFORD, J. 1905 Trematodes from Canadian vertebrates. Zool. Anz. **28**: 681–694.

EXPLANATION OF PLATES

AS, anterior sucker; PPG, peripharyngeal glands; VIT, vitellaria; AC, acetabulum; TES, testis; INT, intestinal caeca; OV, ovary; SR, seminal receptacle; EX, excretory vesicle; VD, vitelline duct; CVD, common vitelline duct; OOT, ootype; MG, Mehlis' gland; UT, uterus; GC, germinal cell; STY, styley; PH, pharynx; PPPG, primordia of peripharyngeal glands; ES, esophagus; PG, penetration glands; GP, genital primordium; CT, collecting tubule; MLE, mesodermal lining of excretory vesicle; BP, birth pore; PT, primordium of testis.

PLATE I

FIGURE 1. Young adult of *Glypthelmins pennsylvaniensis* n. sp. from experimentally infected host. Camera lucida drawing from stained specimen. Ventral view.

FIGURE 2. Sexually mature adult of *G. pennsylvaniensis* from naturally infected host. Camera lucida drawing of stained specimen. Ventral view.

FIGURE 3. Enlarged drawing of area of ootype of female reproductive system showing various associated structures and ducts.

FIGURE 4. Eggs of *G. pennsylvaniensis* as seen in the descending limb of the uterus. Camera lucida drawing.

FIGURE 5. Entire youngest mother sporocyst of *G. pennsylvaniensis* enclosing four germinal cells. Drawn from living material observed with phase-contrast.

FIGURE 6. Second-stage mother sporocyst enclosing germinal cells and smaller somatic cells. Drawn from living material observed with phase-contrast.

FIGURE 7. Miracidium of *G. pennsylvaniensis*. Drawn from living material observed with phase-contrast.

PLATE II

FIGURE 8. Third-stage mother sporocyst of *G. pennsylvaniensis* showing cell aggregates (germ balls) rather than individual cells. Drawn from living material observed with phase-contrast.

FIGURE 9. Oldest mother sporocyst of *G. pennsylvaniensis* showing numerous germ balls. Drawn from living material observed with phase-contrast.

FIGURE 10. Young daughter sporocyst of *G. pennsylvaniensis* immediately after rupturing out of mother sporocyst. Drawn from living material observed with phase-contrast.

FIGURE 11. Fully developed daughter sporocyst of *G. pennsylvaniensis* showing germ balls, cercariae, and germinal mass. Drawn from living material observed with phase-contrast.

FIGURE 12. Cercaria of *G. pennsylvaniensis*. Drawn from living material observed with phase-contrast. Ventral view.

FIGURE 13. Stylet of *G. pennsylvaniensis*. Camera lucida drawing.

FIGURE 14. Anterior end of daughter sporocyst of *G. pennsylvaniensis* showing cercaria escaping through birth pore. Drawn from living material observed with phase-contrast.

FIGURE 15. Encysted metacercaria of *G. pennsylvaniensis*. Camera lucida drawing from stained specimen.

PLATE I

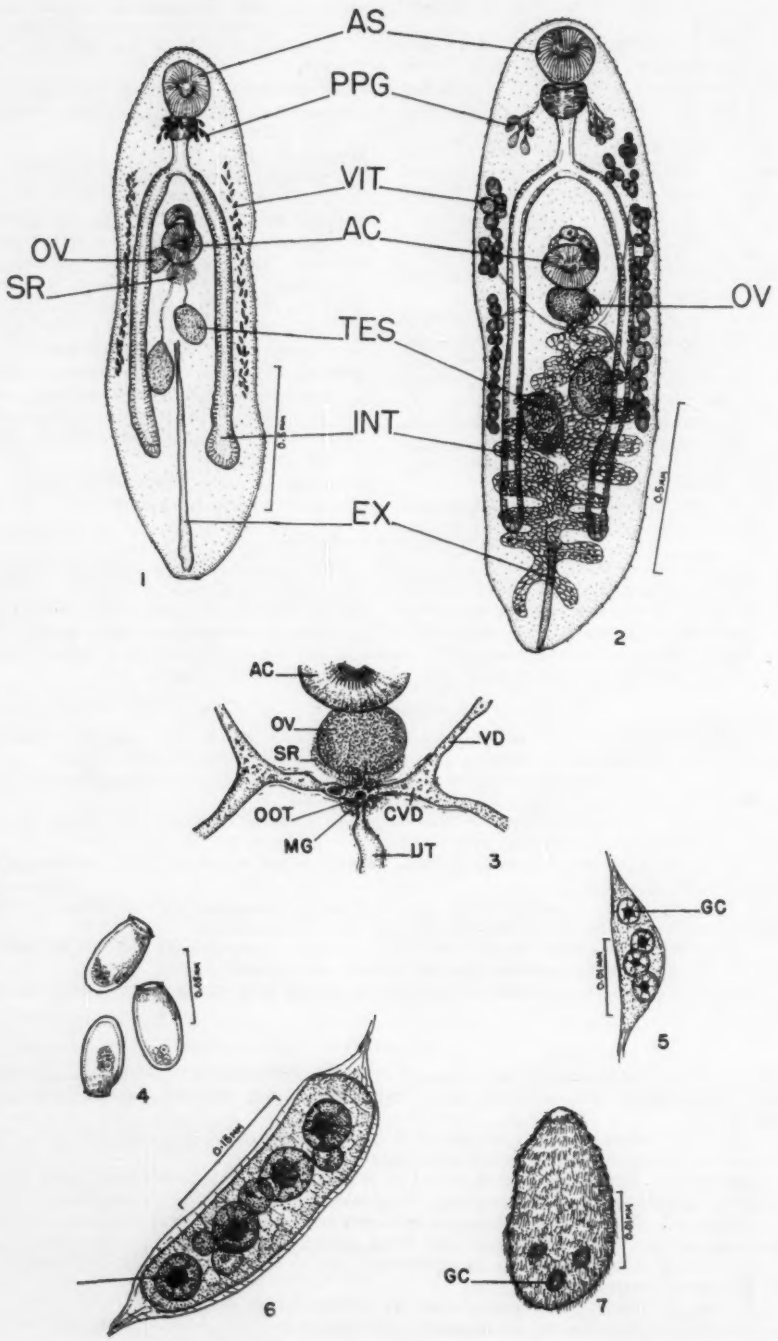
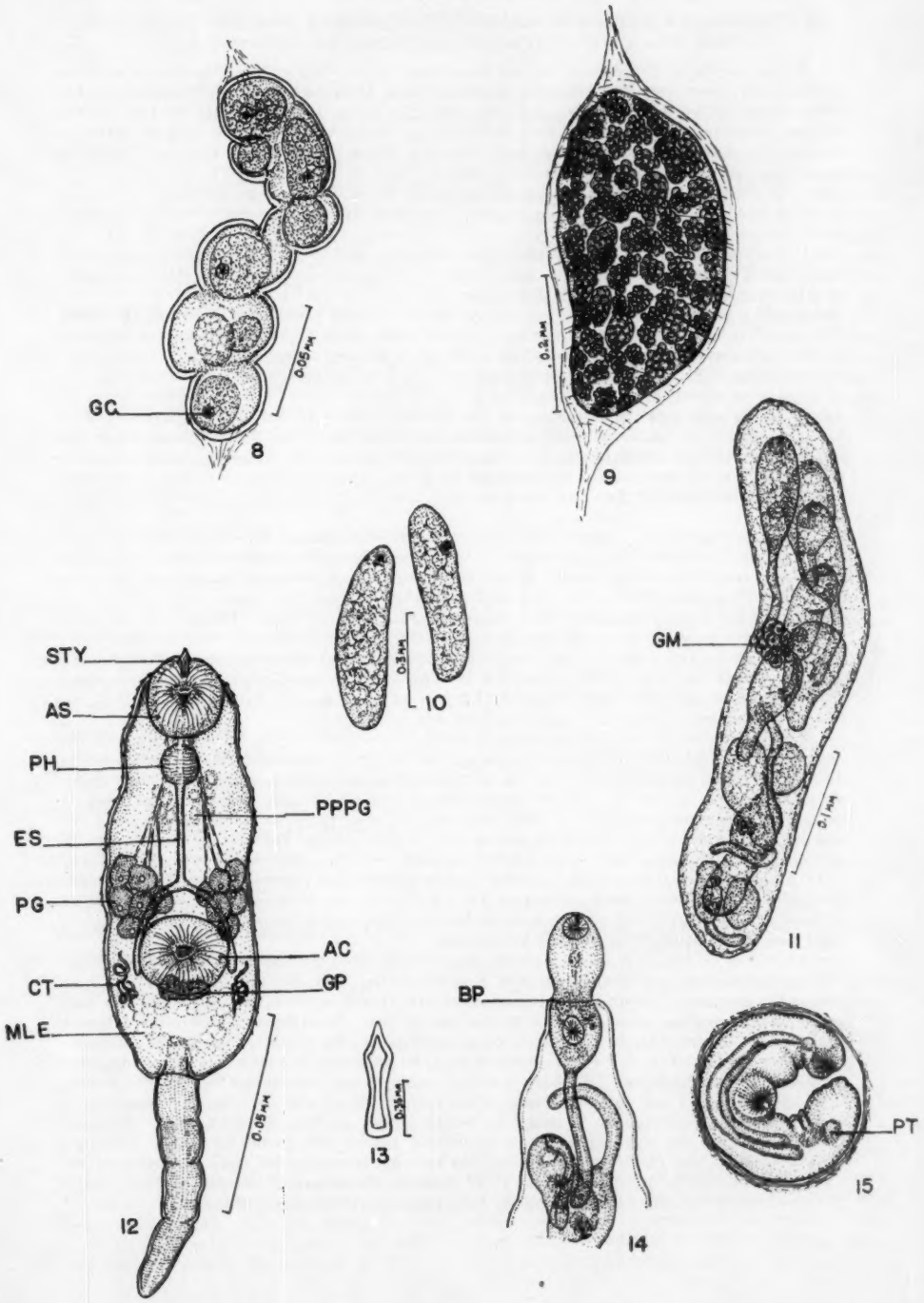

AS
PPG
VIT
AC
OV
SR
TES
INT
EX
OV
1
2
AC
OV
SR
VD
OOT
CVD
MG
UT
3
GC
5
4
6
GC
7

PLATE II


GC
0.05mm
8
0.2mm
9
0.3mm
10
GM
0.1mm
11
STY
AS
PH
ES
PG
CT
ML E
PPPG
AC
GP
0.08mm
12
0.35mm
13
BP
14
PT
15

RESEARCH NOTE

IS THE CILIATE *EUPLOTES BALTEATUS* ADAPTING TO COMMENSAL LIFE IN THE SEA URCHIN *STRONGYLOCENTROTUS DRÖBACHIENSIS*?

In the waters of Frenchman Bay at Mt. Desert Island, Maine, seven species of ciliated protozoa have been reported from the digestive tract of the sea urchin *Strongylocentrotus dröbachiensis* (Powers, 1933, Biol. Bull. **65**: 106–121; Beers, 1948, *Ibid.* **94**: 99–112). Three of them, *Entodiscus borealis, Madsenia indomita,* and *Biggaria gracilis,* are obligate commensals, but the status of the remaining four, *Cyclidium stercoris, Plagiopyla minuta, Trichodina* sp., and a species of *Euplotes,* which the writer (1954, J. Prot. **1**: 86–92) identified as *E. balteatus* (Dujardin), is unclear, owing to inadequate study. Powers regarded them as "chance or vagrant ciliates, which, after being engulfed with food, are able to survive" and to multiply in the host.

Concerning *E. balteatus,* its distribution indicates that it is a facultative commensal, since both Powers and the writer (1954) found it on the nearby algae and other substrata, as well as in the host. To state the facts more accurately, a species of *Euplotes* occurs in the urchin and a morphologically identical species occurs outside; presumably, they are the same. The present note concerns the increasing frequency with which *E. balteatus* has been reported in the urchin at Mt. Desert Island from 1929, when Powers began his study, to 1960. The facts relating to its incidence and density of population in the host follow chronologically.

Although Powers cites the Bay of Fundy as the locality from which his urchins came, he states that he worked in the laboratory of Mr. William Procter of Bar Harbor, Maine, and he indicates in table I that some of his specimens were collected at Mt. Desert Island. Since the town of Bar Harbor is located on Frenchman Bay, which may be regarded as subsidiary to the Bay of Fundy, some of his urchins were no doubt taken in Frenchman Bay. They were very lightly infected with *Euplotes,* since he never found more than 10 to 15 individuals in an urchin.

Eighteen years later (summer 1947) the writer (1948) examined for ciliates a total of 182 specimens of *S. dröbachiensis* (diameter, 3.0 to 6.0 cm). They were collected 3 or 4 at a time at low tide from rocks in the vicinity of the Mt. Desert Island Biological Laboratory, which is located on Frenchman Bay in the town of Bar Harbor, or from the bottom in about 2 m of water near the Laboratory dock. They were examined without delay. Twenty-four of them (13.2 percent) were infected with *Euplotes.* The infections were distinctly heavier than those noted by Powers and amounted to 5 to 10 individuals per 0.1 ml of intestinal fluid. In the summer of 1948 the writer (1954) examined 165 additional urchins from the Laboratory area; the incidence of infection (25 urchins or 15.2 percent) was slightly higher than in 1947, although the number of ciliates per 0.1-ml sample was unchanged.

The passage of 12 more years brought radical increases in both the incidence and the degree of infection with *E. balteatus.* In the summer of 1960 I examined 78 additional urchins (diam., 2.5 to 6.5 cm) from the area; 54 of them (69 percent) were infected. Because of the abundance of the ciliate in many of them, 0.05-ml samples of intestinal fluid were used in estimating the degree of infection. Nine such samples were taken from each urchin, and the number of individuals of *E. balteatus* was counted in each sample. The average number was 28 per sample, representing more than a 5-fold increase over the number observed in 1947 and 1948, and an incalculable increase over the number observed by Powers in 1929. The 3 urchins that were most heavily infected measured 4.0, 2.5, and 5.5 cm in diameter, and contained an average of 140, 131, and 107 specimens of *E. balteatus* per sample, respectively. Dividing specimens were fairly plentiful in many of the urchins.

Thus it is evident that in the past 32 years (1929–1960) *E. balteatus* has become decidedly more abundant in *S. dröbachiensis* in Frenchman Bay, and the records suggest that it is now in the process of adapting to entocommensal life. It is recognized that this conclusion may prove to be erroneous when subjected to the test of time. Nevertheless, it is believed that a record of the observations to date should be incorporated in the literature for future reference.

In conclusion, it is not to be assumed that *E. balteatus* is necessarily becoming more plentiful in *S. dröbachiensis* elsewhere, for this urchin, being circumpolar in northern waters, is widely distributed and its ciliate fauna is not uniform throughout its range. For example, in the San Juan Archipelago of Washington, Berger (1960, J. Prot. **7** (supp.): 17) found *E. balteatus* in only one specimen of *S. dröbachiensis,* but he also found species of *Anophrys, Cryptochilidium,* and *Plagiopyliella,* genera that have not been recorded from the urchin at Mt. Desert Island.—C. D. BEERS, *Department of Zoology, University of North Carolina, Chapel Hill, and the Mt. Desert Island Biological Laboratory, Salisbury Cove, Maine.*

STUDIES ON DIGENETIC TREMATODES OF HAWAIIAN FISHES: FAMILY BUCEPHALIDAE*

H. W. Manter and Mary Hanson Pritchard
University of Nebraska, Lincoln

The following two species of *Dollfustrema* Eckmann, 1934, were among the specimens collected by Dr. Hilda L. Ching during August 1959, at the Waikiki Aquarium. Our previous paper (1960), based on trematodes of Hawaiian eels collected by Pritchard in 1949, included only one gasterostome, *Dolichoenterum microcotylum*.

Yamaguti (1954, 1958) included in the generic diagnosis of *Dollfustrema*, "Rhynchus inverted conical . . . with triple crown of spines . . . Vitellaria divided into paired preovarian groups." This description actually applies only to the type species; in others the rhynchus may be inverted conical or rounded, the crown of spines is multiple (3 to 6), and the vitellaria are either divided into paired preovarian groups or continuous across the body. His key to the genera of Bucephalinae (1958) reflects the restricted description of the rhynchus and its spines. Yamaguti's generic diagnosis of *Dollfustrema* provides for pretesticular or intertesticular ovary, but the subfamily diagnosis (1958) provides only for a pretesticular ovary.

Manter (1940a) once considered *Dollfustrema* a synonym of *Mordvilkovia* Pigulewsky, 1931; but later the same year (1940b) he proposed retention of *Dollfustrema*, assuming the absence of cephalic spines in *Mordvilkovia*. Nagaty (1937) and Yamaguti (1954, 1958) consider *Mordvilkovia* a synonym of *Prosorhynchus* Odhner, 1905.

Measurements of eggs are in microns; others are in millimeters.

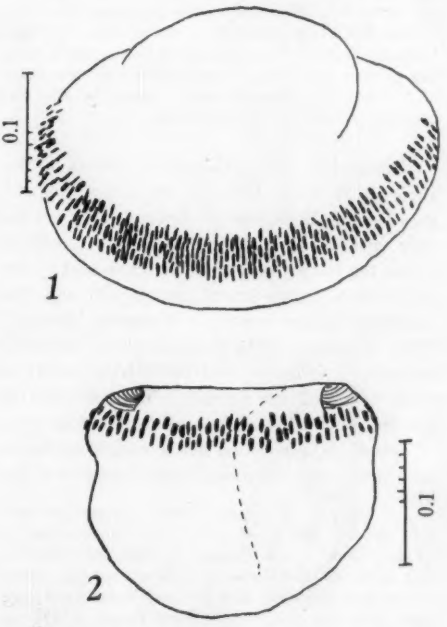
Dollfustrema bipapillosum n. sp.
(Figs. 2–5)

Host: *Gymnothorax petelli* (Bleeker) (Muraenidae, moray eels or puhis); 8 specimens from 1 host.

Location: Intestine.

Holotype: U. S. Natl. Mus. Helm. Coll., No. 39060.

Description (based on 8 specimens): Body oval, rounded anteriorly, tapered and somewhat pointed posteriorly, 1.253 to 1.561 long by 0.456 to 0.570 wide; spines sparse or lacking (probably lost). Rhynchus (fig. 2) 0.194 to 0.235 long by 0.221 to 0.268 wide, terminal, rounded; with anterior, deep, cup-shaped depression almost filled by a large, lateral lobe (usually left, but right in two specimens); muscular papilla occurring laterally on each side of anterior edge of rhynchus; four rows of inconspicuous spines located near anterior edge of rhynchus (fig. 4). Mouth about one-third body length from posterior end, opposite middle or anterior part of cirrus sac; pharynx globular, 0.134 to 0.154 in diameter; cecum saccular in middle one-third of body, 0.315 to 0.369 long by 0.194 to 0.281 wide.



Figures 1 and 2. Drawn by M.H.P. with aid of camera lucida; value of projected scale indicated in millimeters. 1, *Dollfustrema muraenae* from *Gymonthorax vicinus*, holotype; dorsal view of rhynchus; 2, *D. bipapillosum* from *G. petelii*, paratype; dorsal view of rhynchus.

Received for publication December 22, 1960.
* Studies from the Department of Zoology, University of Nebraska, No. 330. Completion of this study was supported by a grant (G 10667) from the National Science Foundation.

Gonads in more or less transverse, or slightly oblique, row, immediately postequatorial. Testes symmetrical or only slightly oblique, oval, 0.161 to 0.235 long by 0.114 to 0.154 wide, far apart, separated by ovary and cecum; cirrus sac sinistral, 0.375 to 0.469 long by 0.101 to 0.121 wide, rather thick-walled, in posterior one-third of body and extending into middle one-third; internal seminal vesicle descending about one-half length of cirrus sac, then ascending one-third or more, up to all, distance to anterior end of sac, thence turning posteriorly to become the straight pars prostatica; prostatic cells largely filling cirrus sac; genital lobe single; genital atrium about one-fifth to one-fourth length of cirrus sac; genital pore 0.047 to 0.134 from posterior end of body.

Ovary spherical, intertesticular, usually nearer right testis and often partially anterior to testes, 0.114 to 0.147 in diameter; Mehlis' gland postovarian, about same size as ovary; uterus descending almost to genital atrium, ascending sinistral to ovary, more or less filling anterior half of body, then descending to genital atrium; uterine seminal receptacle; vitellaria follicular, in uninterrupted transverse band several follicles wide, close to rhynchus; eggs 21 to 24 by 16 to 19 (transparent eggs 22 to 35 by 19 to 24, collapsed eggs 22 to 30 by 13 to 18). Excretory pore terminal, excretory vesicle extending forward to cirrus sac, sometimes reaching mid-cirrus sac; two well-developed, sinuous tubules extending forward more or less laterally. The name *bipapillosum* refers to the two papillae present on the rhynchus.

Discussion. *D. echinatum* (Komiya & Tajimi, 1941) Yam., 1954; *D. muraenae* Sogandares-Bernal, 1959; and *D. bipapillosum* are the only species of *Dollfustrema* with vitellaria across the body anterior to the testes and cecum, rather than in two lateral groups, although this condition is approached in *D. vaneyi* (Tseng,** 1930) Eckmann, 1934. *D. bipapillosum* is unlike the other species in that the vitellaria do not form a curved row; and the rhynchus has an internal lobe, a pair of papillae, and four rows of spines. *D. echinatum* has a conical rhynchus with three rows of spines, testes posterior to the pharynx, a more anterior mouth, and the cirrus sac does not reach the level of the mouth. *D. muraenae* is most similar and differs chiefly in the details of the rhynchus (shorter; with six rows of spines; more slender spines; and apparent lack of depression, lobes, or papillae). The genital pore is somewhat more anterior and the eggs are slightly wider. *D. muraenae* would be even more similar if further collections of it show oblique as well as symmetrical testes, and demonstrate that the protruded region in the center of the rhynchus (fig. 1) represents the everted base of a shallow cavity.

Examination of the type specimen, kindly loaned by the U. S. National Museum, reveals that *D. muraenae* was actually figured in dorsal view and that the cirrus sac is sinistral. Also, the spines circling the rhynchus are arranged in six horizontal rows (fig. 6) rather than three. The scale shown with the figure of *D. muraenae* should be 0.1 mm rather than 0.5 mm.

Although Sogandares-Bernal (1959) stated that *D. echinatum* had testes side by side and a preequatorial mouth, the original description (Komiya and Tajimi, 1941) stated that the testes may be "situated one directly behind the other" while "in others they are located obliquely." The mouth may be preequatorial (as in K. and T.'s fig. 18), but it may be slightly postequatorial (as in K. and T.'s photomicrograph, fig. 67). *D. echinatum* is the only species with testes posterior to the pharynx.

All species of *Dollfustrema* are from eels (*Gymnothorax* sp.) except two from China, *D. echinatum* and *D. vaneyi*. *D. echinatum* is known only from the muscles of a fresh-water fish where it is progenetic; *D. vaneyi* was immature in the intestine of another fresh-water fish. In both these cases, migratory eels may be involved.

** In spite of the fact that the description of this species appeared under the authorship of "Tseng Shen" (Ann. Parasit. 8: 554–561), the correct name of the original author (according to the "Index-Catalogue of Medical and Veterinary Zoology," part 16, p. 5187) is Shen Tseng, so the authors of *D. vaneyi* should be: (Tseng, 1930) Eckmann, 1934. Eckmann (1932) used "Tseng Shen" but changed it to "S. Tseng" in 1934. The "Tseng Shen, 1930" of Yamaguti (1954, 1958); "(Shen, 1930) Eckmann, 1934" of Hanson (1950); "(Shen, 1930) Eckmann, 1932" of Montgomery (1957); and "(Tseng & Shen, 1930) Eckmann, 1934" of Sogandares-Bernal (1959) are all incorrect.

FIGURES 3–9. Drawn by M.H.P. with aid of camera lucida; value of projected scale indicated in millimeters. Abbreviations used: ex, excretory system; gl, genital lobe; p, papilla; sv, seminal vesicle; ut, uterus; vt, vitellaria; 3, *D. bipapillosum* from *G. petelli*, holotype; ventral view; 4, *D. bipapillosum*, holotype; spines of rhynchus: a, ventral view; b, dorsal view; 5, *D. bipapillosum*, holotype; ventral view of cirrus sac; 6, *D. muraenae*, holotype; spines of rhynchus: a, ventral view; b, dorsal view; 7, *D. stromborhynchum* from *G. petelli*, syntype; ventral view; 8, *D. stromborhynchum*, syntype; a, anterolateral view of rhynchus; b, spines of rhynchus; 9, *D. stromborhynchum*, syntype; ventral view of cirrus sac.

Dollfustrema stromborhynchum n. sp.

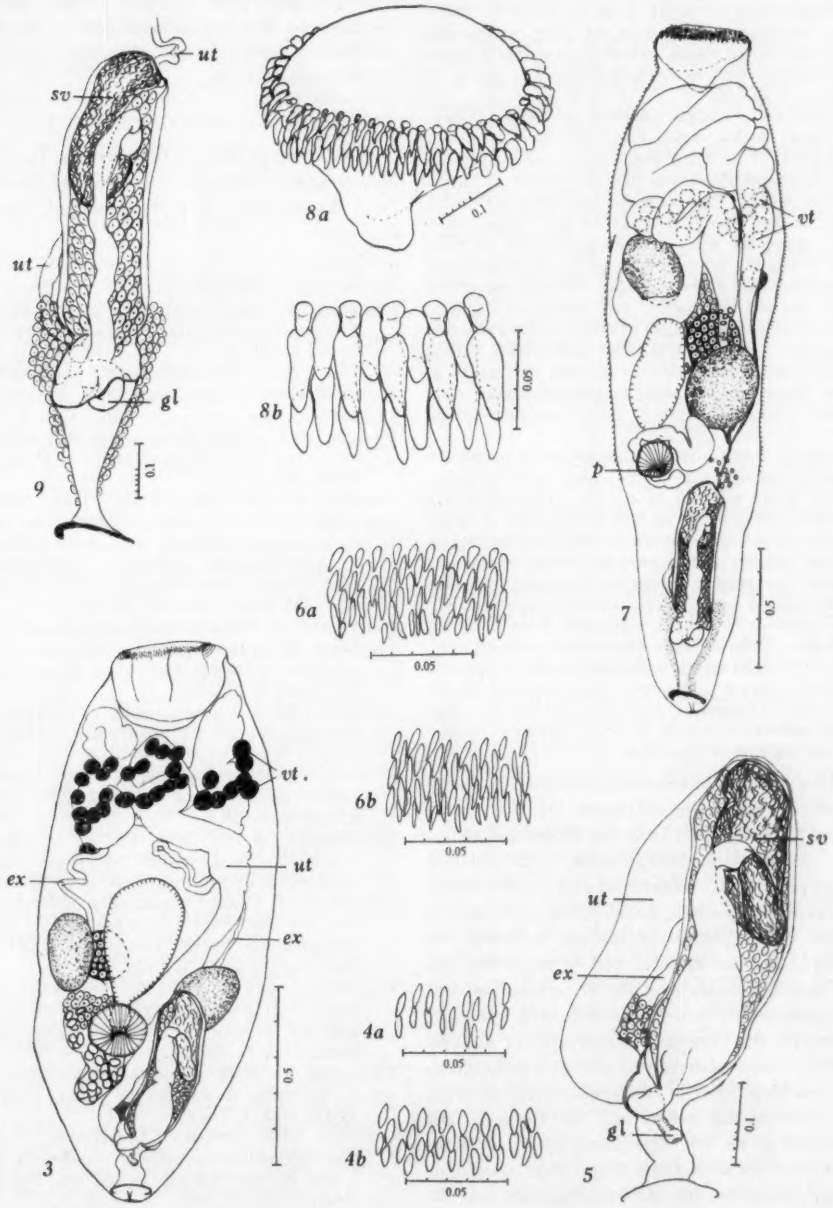
(Figs. 7–9)

Host: *Gymnothorax petelli* (Bleeker) (Muraenidae, moray eels or puhis); 2 specimens from 1 host.

Location: Intestine.

Syntypes: U. S. Natl. Mus. Helm. Coll., No. 39061.

Description (based on 2 specimens): Body elongate, 2.700 to 2.854 long by 0.509 to 0.650 wide, rounded anteriorly, tapering from near midbody and rather pointed posteriorly; body spines short, decreasing in size and density from rhynchus



to level of mouth, scattered posterior to mouth. Rhynchus (fig. 8a) 0.288 to 0.322 long by 0.308 to 0.335 wide, terminal, conical, composed of both muscular and glandular tissue, with 4 rows of relatively large spines and one row of smaller spines (fig. 8b). Mouth one-third body length from posterior end, opening directly into pharynx; mouth overhung anteriorly by oral papilla; pharynx globular, 0.121 to 0.141 long by 0.141 to 0.154 wide; esophagus 0.088 to 0.104 long, surrounded by gland cells; cecum extending anteriorly from pharynx one-third to one-half distance to posterior edge of rhynchus.

Gonads in middle one-third of body, in diagonal row. Testes rounded to oval, 0.288 to 0.415 long by 0.221 to 0.268 wide, one on either side of body with anterior testis dextral; cirrus sac more or less median, in posterior one-third of body, 0.670 to 0.683 long by 0.141 to 0.147 wide, with thick, muscular wall, extending forward to near level of mouth; internal seminal vesicle descending in anterior half of sac, turning forward, ascending about one-half its length; pars prostatica straight, about three-fourths length of cirrus sac, surrounded by prostatic cells; genital lobe single, long, folded at base of genital atrium; genital atrium long, about one-third or more length of cirrus sac; genital pore 0.067 to 0.101 from posterior end of body.

Ovary intertesticular, almost median, mostly dorsal to testes, 0.225 to 0.261 long by 0.201 wide; Mehlis' gland anterior to ovary; uterus extending anteriorly from ovary on left, filling most of space between ovary and rhynchus, then descending to genital atrium; initial part of uterus serving as seminal receptacle; vitellaria follicular, forming uninterrupted band several follicles wide immediately anterior to gonads, separated from rhynchus by loops of uterus; eggs near ovary (transparent) 26 to 30 by 19 to 21, collapsed (yellow) eggs 22 to 26 by 13 to 16. Excretory pore terminal, excretory vesicle extending forward to cirrus sac. The name *stromborhynchum* is from *strombo* (=top) for the top-shaped rhynchus.

Discussion. *D. stromborhynchum* is a fourth species in which the vitellaria are distributed across the body anterior to the cecum and testes. It is similar to *D. bipapillosum* (with which it is sympatric), *D. echinatum*, and *D. muraenae*. The conical rhynchus, the elongate body, the location of the vitellaria midway between the pharynx and the anterior end of the body, and the diagonal testes are like *D. echinatum*, but *D. stromborhynchum* differs by having 5 rows of spines on the rhynchus, larger spines, an oral papilla, testes anterior to the pharynx, cirrus sac reaching level of pharynx, ovary between the levels of the testes, and the vitellaria not arranged in an arc. Specimens of *D. stromborhynchum* are three times longer than specimens of *D. echinatum*, but the latter species was described from a metacercaria.

The band of vitellaria, testes anterior to the pharynx, and pharynx at or very near anterior end of the cirrus sac are like *D. bipapillosum* and *D. muraenae*, but *D. stromborhynchum* differs in its elongate body, conical rhynchus with 5 rows of spines, the vitellaria midway between the pharynx and the anterior end of the body, one testis extending anterior to the cecum, and longer genital atrium.

SUMMARY

Two new species of *Dollfustrema*, *D. bipapillosum* and *D. stromborhynchum*, are described from the intestine of a Hawaiian eel, *Gymnothorax petelli*.

REFERENCES CITED

ECKMANN, F. 1932 Beiträge zur Kenntnis der Trematodenfamilie Bucephalidae. Ztschr. Parasitenk. **5**: 94–111.

———— 1934 Rectifications de nomenclature (*Dollfustrema* nom. nov.). Ann. Parasit. **12**: 256.

HANSON, M. L. 1950 Some digenetic trematodes of marine fishes of Bermuda. Proc. Helm. Soc. Wash. **17**: 74–89.

KOMIYA, Y. AND TAJIMI, T. 1941 Metacercariae from Chinese *Pseudorasbora parva* Temminck and Schlegel with special reference to their excretory system. I. (Metacercariae from Chinese fresh waters No. 1). J. Shanghai Sci. Inst. N.S. **1**: 69–106, 19 pls.

MANTER, H. W. 1940a Digenetic trematodes of fishes from the Galapagos Islands and the neighboring Pacific. Rep. Allan Hancock Pacific Exped. **2**: 325–497.

———— 1940b Gasterostomes (Trematoda) of Tortugas, Florida. Papers Tortugas Lab. **33**: 1–19.

———— AND PRITCHARD, M. H. 1960 Some digenetic trematodes of eels of Hawaii. J. Parasit. **46**: 651–658.

MONTGOMERY, W. R. 1957 Studies on digenetic trematodes from marine fishes of LaJolla, California. Trans. Am. Micr. Soc. **76**: 13–36.

NAGATY, H. F. 1937 Trematodes of fishes from the Red Sea. Part I. Studies on the family Bucephalidae Poche, 1907. Publ. (12) Fac. Med. Egypt. Univ. 172 pp.

SOGANDARES-BERNAL, F. 1959 Digenetic trematodes of marine fishes from the Gulf of Panama and Bimini, British West Indies. Tulane Stud. Zool. **7**: 69–117.

YAMAGUTI, S. 1954 Systema Helminthum. Part I. Digenetic trematodes of fishes. Published by the author. Tokyo.

———— 1958 Systema Helminthum. Vol. 1. The digenetic trematodes of vertebrates. Parts I and II. Interscience Publishers, Inc., New York.

STUDIES ON DIGENETIC TREMATODES OF HAWAIIAN FISHES: FAMILIES MONORCHIIDAE AND HAPLOPORIDAE*

H. W. Manter and Mary Hanson Pritchard
University of Nebraska, Lincoln

Some of the following trematodes were collected in 1949 by one of us (M.H.P.); others by Hilda L. Ching in 1959. The fishes were identified by Dr. William A. Gosline. All measurements are in millimeters except those of eggs which are in microns.

FAMILY MONORCHIIDAE

The taxonomy of the family Monorchiidae is in a state of considerable uncertainty. Its division by Yamaguti (1958, p. 61) into 10 subfamilies is highly artificial. This system places in different subfamilies such closely related genera as *Monorcheides* and *Paramonorcheides*, (Szidat, 1950, considered these synonymous), or *Hurleytrema* and *Hurleytrematoides*. Minor differences in the distribution of the vitellaria should not be of more than generic value; in some cases, only specific. Martin (1940) suspects the Y-shaped excretory vesicle described for some monorchids is probably a median vesicle receiving expanded vessels; therefore, this character should not be stressed in this family. Perhaps it is not advisable to attempt subfamily categories as yet. If a division is desired, we would suggest the following five subfamilies.

Subfamily Monorchiinae: Genital pore median or submedian, usually preacetabular; cirrus, cirrus sac, metraterm, terminal organ, and genital atrium present and at least one, perhaps all, spined; testes one or two; eggs without filament. Genera: *Monorchis* Looss, 1902; *Proctotrema* Odhner, 1911; *Lasiotocus* Looss in Odhner, 1911; *Monorcheides* Odhner, 1905; *Genolopa* Linton, 1910 (syn. *Paraproctotrema* Yam., 1934); *Proctotrematoides* Yam., 1938; *Paramonorcheides* Yam., 1938; *Diplomonorcheides* Thomas, 1959; *Allolasiotocus* Yam., 1959; *Postmonorchis* Hopkins, 1941; *Postmonorcheides* Szidat, 1950; *Telolecithus* Lloyd & Guberlet, 1932; *Pseudoproctotrema* Yam., 1942; *Botulisaccus* Caballero, Bravo & Grocott, 1955; *Ametrodaptes* Bravo, 1956.

Subfamily Asymphylodorinae: Genital pore lateral or sublateral, near midacetabular level. Testes one or two. Genera: *Asymphylodora* Looss, 1899; *Palaeorchis* Szidat, 1943; (?) *Brahamputrotrema* Dayal & Gupta, 1954.

Subfamily Hurleytrematinae: Eggs filamented. Testes one or two. Genera: *Hurleytrema* Srivastava, 1939; (syn. *Pseudohurleytrema* Yam., 1954); *Hurleytrematoides* Yam., 1954; *Diplolasiotocus* Yam., 1952.

Subfamily Opisthomonorchiinae. Genital pore median, postacetabular; testis one. Genus: *Opisthomonorchis* Yam., 1952.

Subfamily Octotestiinae Yam., 1958. Eight testes. Genus: *Octotestis* Yam., 1951.

COMMENTS ON CERTAIN GENERA OF MONORCHIIDAE

We prefer to retain *Botulisaccus* in the Monorchiidae. Yamaguti (1958) placed it in the Zoogonidae, but the spined cirrus, posterior cirrus sac, and only slightly lateral genital pore are more like monorchids. The Zoogonidae is probably a related family. The sac-like structure near the end of the uterus in *Neozoogonus californicus* Arai, 1954 may be a homolog of the "terminal organ" of monorchids.

Yamaguti included *Triganodistomum* Simer, 1929 in the Monorchiidae (with some uncertainty), but this genus is a close relative, if not a synonym, of *Lissorchis* Magath, 1916 (Family Lissorchiidae).

Most troublesome of monorchid genera have been *Proctotrema* Odhner, 1911, "*Lasiotocus* Looss, 1907," and *Genolopa* Linton, 1910. We believe recent authors have erred in accepting the validity of *Lasiotocus* as of Looss, 1907, and in recognizing the genus "*Pristisomum*." As Odhner (1911, p. 250, footnote) has noted, these names of Looss were not seriously proposed but were merely simulated or suppositious names. Looss (1907, p. 616) was indignant that the Rules of Nomenclature permitted the creation of new generic names merely by referring to some type species. He then tried to show how absurd it would be if (for example) he should give the name *Lasiotocus* to *Dist. mulli* Stoss., 1883 as

Received for publication December 30, 1960.

* Studies from the Department of Zoology, University of Nebraska, No. 327. Completion of these studies was supported by a grant (G10667) from the National Science Foundation.

type. We take the view that when Looss stated: "Wenn ich endlich schreiben würde *Lasiotocus* n. gen., typus *Dist. mulli* Stoss." that he was not actually naming a genus. Odhner (1911) did not consider these names as having priority from 1907 but he did recognize them as "Looss in mnscr.". However, the manuscript was never published. Dollfus (1948) reviewed the above history and decided to accept the generic names because a known type species was specified. Such a recognition would make Looss guilty of a practice he deplored. Dollfus described in detail the type species of *"Lasiotocus," Dist. mulli* Stoss. We agree with Odhner that these generic names cannot be valid from the 1907 date, because Looss did not, in fact, give names to these genera. Perhaps he intended to validate them with full descriptions at a later date.

Several authors recently have considered *Proctotrema* and *Lasiotocus* to represent a single genus. If so, as maintained above, *"Lasiotocus* Looss, 1907" is not a name until established as "Looss in manscr." by Odhner in 1911. In this case, *Proctotrema* Odhner, 1911 would have page priority. However, we believe that *Proctotrema* (type species *bacilliovatum* Odhner, 1911) can be separated from *Lasiotocus* on the basis of a 3- or 4-lobed ovary, a character usually correlated with an elongate oral sucker. *Proctotrema,* in this sense, would include: *P. bacilliovatum* Odhner, 1911; *P. macrorchis* Yam., 1934; *P. plectorhynchi* Yam., 1934; *P. chaetodipteri* (Thomas, 1959) n. comb. (syn. *Lasiotocus chaetodipteri*); *P. himezi* (Yam., 1951) n. comb. (syn. *Lasiotocus himezi*); *P. parvum* Manter, 1942; *P. latum* (Manter, 1942) n. comb. (syn. *Monorchis latus*). We believe a muscular terminal portion of the uterus varies too much in degree to determine a genus. Such a structure is fairly well developed, but undescribed, in *Lasiotocus truncatus* (Linton, 1910). The genus *Monorchis* should be limited to species with preacetabular vitellaria. The ovary of *M. monorchis,* according to Timon-David (1937) and Vlasenko (1931), is irregular in outline, not distinctly lobed. Only two species now would fall into *Monorchis: M. monorchis* (Stoss., 1890) Looss, 1902, and *M. parvus* Looss, 1902.

The generic name *Lasiotocus* becomes available, we believe, as *Lasiotocus* Looss in Odhner, 1911, with *L. mulli* as type species. The genus resembles *Proctotrema* except that the ovary is unlobed, although rarely slightly irregular in shape. The genital atrium is unspined.

The genus *"Pristisomum"* Looss, 1907, was even more imaginary than *"Lasiotocus."* It seemed to be based on an imaginary species *"pumex"* from, however, an actual fish (*Caranx trachurus*). *"Pristisomum"* not only has no more status than *"Lasiotocus"* but in addition it is (or would be) a *nomen nudum* for it is based on a simulated species still undescribed. The only character Looss mentioned was "spined copulatory organs," a character occurring in several families. Odhner (1911) had no authority to characterize this genus. Yamaguti (1954, 1958) inexplicably lists *Postmonorchis* Hopkins, 1941, as a synonym of *Pristisomum,* and his generic diagnosis must be based on *Postmonorchis.*

The genus *Genolopa* Linton, 1910, has had a varying status and is still a rather obscure genus. Manter (1942) and Thomas (1959) have proposed that it be separated from *Lasiotocus* (= *Proctotrema*) on the basis of its spined genital atrium. In the type species, *G. ampullacea,* there are three distinct types of spines situated respectively in the cirrus, metraterm, and atrium. Thomas recognized three species in *Genolopa: G. ampullacea* Linton, 1910; *G. fusiforme* (Yam., 1934); and *G. lethrini* (Yam., 1953). The genus *Proctotrematoides* Yam., 1953, was accepted by Thomas on the basis of the atrial diverticle and the long excretory vesicle. We accept this genus with some hesitation because the atrial diverticle is probably variable; the posterior shallow atrial sac in *G. ampullacea* probably represents it. A species of *Genolopa* described below has no spines in the metraterm and only rudiments of spines on the cirrus sac. Therefore, the atrial spines may be the chief generic character. *G. trifolifer* Nicoll, 1915, showed no spines in any of the terminal ducts and, for the present, must remain of uncertain status. *Paraproctotrema brevicaecum* Manter, 1942, has no spines in the metraterm and few in the cirrus. Those which appear to be in the atrium were interpreted to be on the tip of an everted cirrus, but neither the figure nor two paratype specimens favor this interpretation. Thus, this species becomes *Genolopa brevicaecum* (Manter, 1942) n. comb. Another species in the genus is *G. bupharynx* Bravo-Hollis, 1956.

Admittedly, the presence of atrial spines is sometimes difficult to discern, particularly if the cirrus or metraterm is everted, but no better character seems available to separate certain

species from *Lasiotocus*.

The uterus of monorchids connects with a peculiar sac-like structure lying alongside the cirrus sac. It has been called the terminal organ, the vaginal sac, and the metraterm sac. Of these terms the first seems preferable. The organ usually consists of a swollen base containing a vesicle, and a distal tubular portion; either one or both these regions may be spined. The vesicle usually contains some flattened cells in its lumen; its function is problematical. Only rarely have we seen any eggs or typical sperm cells in the terminal organ. The uterus usually enters the terminal organ near its middle just anterior to the vesicle; in some species the uterus enters the posterior end of the organ. The distal tubular portion of the terminal organ is referred to as a metraterm. In many monorchids, the terminal part of the uterus, just before it enters the terminal organ, has a muscular wall, the "muscular bulb" of Yamaguti. This metraterm-like structure, perhaps the true metraterm, is never spined.

Genolopa oculata n. sp.
(Figs. 1–2)

Host: *Aubula vulpes* (L.) 'o'io (Albulidae, ladyfish; bonefish); 10 specimens from 1 host.

Location: Intestine.

Holotype: U. S. Natl. Mus. Helm. Coll., No. 39466.

Description (measurements on two): Body spined, 1.206 to 1.212 by 0.355 to 0.368; more broadly rounded at posterior end; conspicuous, definite, asymmetrical eyespots in forebody. Oral sucker 0.104 in diameter; acetabulum very weakly developed, 0.064 to 0.072 in diameter; sucker ratio about 1: 0.65 to 0.7. Forebody about 0.355 or a little more than one-fourth body length. Prepharynx short; pharynx spherical, 0.042 to 0.049 in diameter; esophagus a little longer than pharynx; bifurcation nearer oral sucker than to acetabulum; ends of ceca not visible in most specimens but apparently slightly posterior to testis. Testis, elongate-oval, near middle third of hindbody. Cirrus sac curving around acetabulum to level of ovary; containing an ovoid seminal vesicle, conspicuous prostatic cells, and a short, muscular, unspined cirrus; terminal portion of cirrus sac protruded into atrium (fig. 2), functioning as a cirrus, its surface sometimes with large, plate-like rudiments of spines. Genital pore just preacetabular, submedian. Genital atrium swollen by protrusion of cirrus sac, with few to many scattered spines. Ovary large, subspherical, partly dextral and partly anterior to testis. Vitellaria of large follicles, 6 to 8 on each side from level of acetabulum to anterior edge of ovary or slightly beyond. Uterus filling posttesticular space, entering middle of terminal organ; metraterm a short, weakly muscular, ovoid or pyriform in shape and unspined. Terminal organ about 0.234 long, or little shorter than cirrus sac. Eggs rather thick-shelled, 24 to 27 by 14 to 16 microns. Excretory vesicle not traced, probably short.

Discussion: This species differs from most others in the genus in having unspined terminal organ and mostly unspined cirrus sac and cirrus, although it does resemble *G. brevicaecum* in these respects. *G. brevicaecum* differs in sucker ratio, its much longer esophagus, shorter ceca, smaller size, and smaller eggs.

Monorcheides decapteri n. sp.
(Figs. 3–4)

Host: *Decapterus pinnulatus* (Eydoux & Souleyet), opelu (Carangidae); 1 specimen in 1 of 3 hosts.

Location: Intestine.

Holotype: U. S. Natl. Mus. Helm Coll., No. 39465.

Description: Length 1.192; greatest width near midbody, 0.435; body tapering toward each end to rounded point. Body spines lost; eye spots not evident. Oral sucker 0.08 wide; acetabulum weakly developed, just behind midbody (forebody 0.603), 0.077 wide; sucker ratio 1: 0.96, or subequal. Pharynx small and weak, spherical, 0.034 in diameter; prepharynx slightly longer than pharynx; esophagus long and slender, about 0.25 long; ceca little divergent, slender, extending to near end of cirrus sac on one side, or little past cirrus sac on other. Left testis very long, 0.335 by 0.1, to left of midline extending to near posterior end of body. Right testis not clear, degenerate, testicular cells in region opposite left testis. Cirrus sac large, curved, its proximal end touching anterior fourth of testis, containing an ovoid seminal vesicle, numerous prostatic cells, and spined cirrus; cirrus less than one-third length of cirrus sac; posterior (or mesial) spines long and slender, anterior (or outer) spines thorn-shaped (fig. 4). Ovary irregular in outline, extended laterally, almost bilobed, in middle fourth of body, to right of left testis, overlapping base of cirrus sac. Vitelline follicles ovoid to elongate, in middle fourth of body, about 20 follicles on left extending to midline and overlapping acetabulum, about 9 follicles on right side; vitelline ducts large, meeting a little posterior to ovary; seminal receptacle not seen, probably lacking as in most monorchids. Uterus not extending posterior to testes, overlapping left testis ventrally, with relatively few eggs. Eggs 19 to 21 by 11 to 14 microns. Terminal organ with subspherical vesicle, to left of curve of cirrus sac, spined with needle-like spines which continue into metraterm. Genital pore inconspicuous, median, just anterior to acetabulum. Spines in genital atrium few. Excretory pore ventral, subterminal, stem very short; vesicle apparently Y-shaped.

Discussion: The genera *Monorcheides* and *Paramonorcheides* are very similar except for the shape of the excretory vesicle, supposedly V- or Y-shaped in *Monorcheides* and I-shaped or sac-

shaped in *Paramonorcheides*. Among most trematodes, this difference would be important, but Martin has suggested (1940) that in this family the collecting vessels near the vesicle may swell to resemble forks of the vesicle itself. In *Paramonorcheides* much of the uterus is posttesticular; in *Monorcheides*, all or most of the uterus is pretesticular.

M. decapteri differs from all other species in the genus by its very long forebody and esophagus. In spite of the probable occurrence of two testes in *M. decapteri*, the species shows considerable similarity to *Telolecithus tropicus* Manter, 1940 from a related host from the west coast of Panama. In both species there is a long forebody and esophagus, elongate testis, irregularly shaped ovary, uterus not extending posterior to testis, spined metraterm and cirrus, and Y-shaped excretory vesicle.

Yamaguti (1958) moved *T. tropicus* to the genus *"Pristisomum"* which he considered the equivalent of *Postmonorchis* Hopkins, 1941. As noted above, we do not accept the genus *Pristisomum*. *T. tropicus* has a number of differences from other species of *Postmonorchis* and should be placed in a separate genus to which we give the name *Chrisomon* (masculine, derived as an anagram from the word *Monorchis*).

Diagnosis of Chrisomon: Monorchiinae. Body elongate; esophagus more than twice length of pharynx; testes single, elongate, near posterior end of body; cirrus and metraterm spined; ovary irregularly lobed; vitellaria of numerous follicles, not reaching acetabulum; uterus not extending posterior to testis; excretory vesicle apparently Y-shaped with very short stem. Type species: *C. tropicus* (Manter, 1940) n. comb. (syn. *Telolecithus tropicus*).

Lasiotocus delicatus n. sp.
(Figs. 5–7)

Hosts: Naso sp. (either *N. unicornis* (Forskål) or *N. brevirostris* (Cuv. & Val.)), type host (Acanthuridae, surgeonfishes); 2 specimens from 1 of 28 hosts. *Mulloidichthys auriflamma* (Forskål), weke-'ula (Mullidae, goatfishes); 2 specimens from 1 of 6 hosts. *Parupeneus multifasciatus* (Quoy & Gaimard), moano (Mullidae, goatfishes); 14 specimens from 7 of 29 hosts. *P. pleurostigma* (Bennett), malu (Mullidae, goatfishes); 1 specimen from 1 of 6 hosts. *P. porphyreus* (Jenkins), kumu (Mullidae, goatfishes); 6 specimens from 1 of 4 hosts.

Location: Intestine.

Holotype: U. S. Natl. Mus. Helm. Coll., No. 39469.

Description (measurements on 3 specimens): Body delicate; spined; 1.237 to 1.809 by 0.321 to 0.402; forebody long and slender 0.549 to 0.938 or about one-half body length; posterior end rounded; eye spots lacking. Oral sucker 0.080 to 0.100 in diameter; acetabulum 0.100 to 0.120; sucker ratio 1: 1.12 to 1.5. Prepharynx short; pharynx 0.056 to 0.062 long by 0.052 to 0.060 wide; esophagus very long, bifurcating just anterior to genital pore; ceca ending opposite anterior half of testis or up to a point slightly beyond testis, not reaching posterior to uterus. Genital pore median or submedian, a little anterior to acetabulum. Testis in posterior half of hindbody, somewhat longer than wide; cirrus sac large, claviform, 0.308 to 0.502 long by 0.064 to 0.112 wide, containing an ovoid seminal vesicle, well developed prostatic cells of two kinds (more posterior, transparent cells opening into short pars prostatica, more anterior cells lead to tip of cirrus), and long cirrus armed with large thorn-shaped spines (fig. 7); distal tip of cirrus sometimes evaginated into atrium. Ovary ovoid, unlobed, immediately anterior to and sometimes slightly overlapping testis. Vitelline follicles lateral, few, elongate, from level of acetabulum to ovary or anterior end of testis; uterus extending posterior to testis but not quite to posterior end of body. Terminal organ 0.241 to 0.308 long or not quite as long as cirrus sac. Uterus narrows to a muscular tube with a slightly swollen terminal region which enters middle of terminal organ. Metraterm armed with long, fine, needle-shaped spines; basal vesicle of terminal organ 0.048 to 0.087 wide, unspined. Genital atrium unspined. Eggs 16 to 17 by 8 to 10 microns. Excretory pore subterminal, ventral. Excretory vesicle short.

Discussion: The name *"delicatus"* refers to the soft, delicate condition of the adults most of which, although freshly collected, were somewhat macerated. The forebody is very thin and was often folded when the specimens were killed.

After the revisions proposed above, the genus *Lasiotocus* includes the following species: *L. mulli* (Stoss.); *L. beauforti* Hopkins, 1941; *L. cacuminatus* (Nicoll, 1915); *L. cynoglossi* Thomas, 1959; *L. costaricae* (Manter, 1940); *L. elongatus* (Manter, 1931); *L. lintoni* (Manter, 1931); *L. longovatus* (Hopkins, 1941); *L. longicaecus* (Manter, 1940); *L. malasi* (Nagaty, 1948); *L. minutus* (Manter, 1931); *L. odhneri* (Srivastava, 1939); *L. truncatus* (Linton, 1910).

The only one of these species with non-funnel shaped oral sucker and with esophagus much longer than the pharynx is *L. mulli;* it differs from *L. delicatus* in that the esophagus is only twice the length of the pharynx, the eggs are much larger, and the spines of the cirrus are different. *L. cacuminata* has an esophagus only twice the length of the pharynx, a funnel-shaped oral sucker, and the vitellaria are posterior to the cirrus sac.

Lasiotocus longicaecus described from *Anisotremus interruptus* from the Galapagos Islands and from *A. virginicus* at Tortugas, Florida, is also known from *A. davidsonii* at La Jolla, California, and from *Lethrinus atlanticus* from Ghana, Africa (Thomas, 1959). We also have specimens collected from *A. pacificus* by A. O. Foster in the Miraflores Locks, Panama Canal. We can confirm Thomas' observation on the extent of the excretory vesicle to the ovary or almost to the acetabulum. This species has a very wide distribution in related fishes from tropical African Atlantic to the Gulf of Mexico, to the Panamanian Pacific, Galapagos

Islands, and north to La. Jolla, California.

Hurleytrematoides coronatum n. sp.
(Figs. 8–11)

Hosts (chiefly Chaetodontidae, butterfly fishes): *Chaetodon lineolatus* (Cuv. & Val.), type host; 16 specimens from 5 of 6 hosts. *C. auriga* Forskål; 8 specimens from 4 of 28 hosts. *C. fremblii* Bennett; 70 specimens from 11 of 17 hosts. *C. lunula* (Lacépède); 15 specimens from 5 of 10 hosts. *C. miliaris* Quoy & Gaimard; 19 specimens from 10 of 43 hosts. *Naso hexacanthus* (Bleeker) (Acanthuridae, surgeonfishes); 1 specimen from 4 hosts.

Location: Intestine.

Holotype: U. S. Natl. Mus. Helm Coll., No. 39467.

Description (based on 30 specimens): Body elongate, 0.804 to 1.956 long by 0.168 to 0.422 wide, usually widest near midbody; cuticula bearing rows of small, closely set, triangular spines decreasing in size and number on posterior half of body. Oral sucker subterminal, rounded, 0.088 to 0.176 wide by 0.073 to 0.133 long; acetabulum one-fourth to one-half body length from anterior end, 0.056 to 0.144 wide by 0.048 to 0.120 long; sucker ratio 1: 0.62 to 0.87. Prepharynx short or not apparent; pharynx rounded, 0.051 to 0.104 long by 0.051 to 0.110 wide; esophagus slender, 0.072 to 0.168 long; cecal bifurcation near middle of forebody, ceca terminating posterior to testis in posterior third of body length.

Testis near midbody, median, ovoid to rounded, 0.128 to 0.302 long by 0.080 to 0.221 wide. Cirrus sac pyriform, preovarian, more or less median, 0.269 to 0.440 long by 0.040 to 0.104 greatest width, protruding from genital pore by 0.045 to 0.112. Cirrus sac containing bipartite seminal vesicle, prostatic cells, prostatic duct, and spined cirrus 0.040 to 0.080 long by 0.010 to 0.016 wide. Tip of cirrus evaginable (fig. 9). Genital pore median, preacetabular, between cecal bifurcation and acetabulum; atrium relatively shallow, well filled by protruded part of cirrus sac.

Ovary subglobular, immediately pretesticular, median or submedian, somewhat more ventral than testis, 0.093 to 0.207 long by 0.064 to 0.144 wide. Few, relatively large vitelline follicles in two lateral groups, extending from level of acetabulum to anterior edge of testis, often overlapping ceca dorsally and ventrally. Individual vitelline ducts joining single large duct leading medianly from each group of follicles, large ducts joining dorsal to ovary, locus of vitelline reservoir. Uterus with several longitudinal loops suggesting figure eights, between ovarian level and posterior end of body; proximal descending limb containing sperm cells; uterus entering vesicle of terminal organ near its posterior end. Terminal organ well developed, beginning near acetabulum and extending forward ventral to cirrus sac and slightly to left of median line; vesicle spherical and unarmed; metraterm 0.088 to 0.176 long by 0.019 to 0.026 wide, with short, slender spines, joining left side of genital atrium. Eggs numerous, yellowish, usually collapsed; opercular end rounded, anopercular end pointed and with filament which becomes increasingly fine toward its distal end; eggs 32 to 45 long by 16 to 26 wide, filament 10 to 15 times length of egg (fig. 11). Excretory pore terminal; excretory vesicle tubular, traced to level of testis.

The species name *"coronatum"* (from Latin *"corona"* = wreath) refers to the manner in which the uterus coils around the testis and ovary.

Variations: The smallest adult specimens (uterus with numerous eggs) are less than half the length of the largest, but there is a continuous gradation between the extremes with 75 percent of the 99 specimens between 1.0 to 1.5 long. Twelve young specimens (very few eggs in uterus) were between 0.9 and 1.2 long, while 14 immature specimens (no eggs in uterus) were between 0.5 and 1.0 long. Short specimens tended to have relatively longer ceca and relatively shorter posttesticular distances. In them, the ceca had peculiar, internal, transverse folds (fig. 10); the cirrus sacs were more slender and the anterior parts of the seminal vesicles more elongate; the testes were more rounded than elongate. Range of egg-size, however, is similar in specimens of all sizes.

Discussion: Srivastava (1939) named *Hurleytrema* for *H. ovocaudatum*. Manter (1942) added *H. chaetodoni* and *H. eucinostomi*, but Yamaguti (1954) designated these as the type species of *Hurleytrematoides* and *Pseudohurleytrema* respectively. Bravo-Hollis (1956) named *Hurleytrema longitestis*, and Thomas (1959) described *Hurleytrema trachinoti*.

The Hawaiian species combines characters to be found in both *Hurleytrematoides* (long ceca, bipartite seminal vesicle) and *Pseudohurleytrema* (lateral vitellaria, terminal organ vesicle), but it differs from either genus in the anterior extent of the vitellaria and the absence of a spined median vesicle of the terminal organ. We recognize *Hurleytrematoides* for species having a bipartite seminal vesicle (*H. chaetodoni* and *H. coronatum*). Since various combinations of characters as well as intermediate conditions exist among the remaining species, we believe it would be better to consider them all *Hurleytrema; Pseudohurleytrema* becoming a synonym of *Hurleytrema*.

H. coronatum is unlike *H. chaetodoni* in that the vitellaria extend in separate lateral groups from the level of the acetabulum to the level of the anterior end of the testis; the cirrus is relatively short and minutely spined; the terminal organ has an unarmed vesicle at its base; the sucker ratio is smaller (1: 0.62 to 0.87 as compared with 1: 1); and the filament of the egg is much longer.

FAMILY HAPLOPORIDAE
Syn: Waretrematidae
Megasolenidae

Hapladena spinosa n. sp.
(Figs 12–14)

Host: *Acanthurus sandvicensis* (Streets), manini or convict tang (Acanthuridae, surgeon

fishes); 31 specimens from 18 of 56 hosts.

Location: Intestine.

Holotype: U. S. Natl. Mus. Helm. Coll., No. 39472.

Description (based on 12 specimens): Body elongate, 2.111 to 4.288 long by 0.549 to 0.734 wide, anterior and posterior ends of body rounded; cuticula about 0.011 thick, with spines extending full length of body but decreasing in density posteriorly (although cuticula apparently eroding easily, part or all of forebody retaining cuticula and spines). Oral sucker subterminal, 0.221 to 0.248 wide by 0.154 to 0.268 long; acetabulum 0.168 to 0.235 wide by 0.141 to 0.241 long, slightly protuberant, one-fourth or less of body length from anterior end; sucker ratio 1: 0.79 to 0.95, usually 0.80 to 0.88. Prepharynx often contracted but reaching 0.080 long; pharynx pyriform, 0.107 to 0.181 long by 0.121 to 0.168 wide; esophagus 0.268 to 0.402 long; cecal bifurcation dorsal to acetabulum, ceca relatively wide and extending to near posterior end of body, blood often present in ceca.

Posttesticular space 0.369 to 1.226; testis elongate, 0.335 to 0.637 long by 0.214 to 0.395 wide, usually with lateral swelling at origin of vas efferens; external seminal vesicle tubular, rather sinuous, beginning midway between ovary and hermaphroditic sac or nearer ovary. Hermaphroditic sac 0.201 to 0.503 long by 0.114 to 0.168 wide, almost entirely dorsal to acetabulum, containing: tubular internal seminal vesicle 0.156 to 0.200 long by 0.040 to 0.083 wide, narrowing to duct 0.064 to 0.096 long by 0.016 to 0.027 wide; duct bending somewhat posteroventrally and joining metraterm; hermaphroditic duct 0.120 to 0.168 long by 0.088 to 0.096 wide, eversible; minute spines, situated in lumen of hermaphroditic duct immediately anterior to entry of seminal vesicle (fig. 13), covering tip of everted duct (fig. 14); prostatic complex inconspicuous. Genital pore median, 0.047 to 0.112 anterior to acetabulum, surrounded by well-developed sphincter (fig. 13).

Ovary 0.034 to 0.188 anterior to testis, median, rounded or subglobular, 0.174 to 0.281 long by 0.174 to 0.268 wide; Mehlis' gland immediately anterior to ovary, well developed, usually larger than ovary; Laurer's canal well developed, pore dorsal and right of midovary, canal with irregular swellings extending anteroventrally; seminal receptacle ventral to Mehlis' gland, 0.176 to 0.273 long by 0.088 to 0.121 wide, usually bent in middle. Vitellaria follicular, but follicles often elongate and sometimes joined or branched; follicles extending from near posterior edge of acetabulum to tips of ceca near posterior end of body; peripheral (dorsal, ventral, and lateral) except immediately ventral to ovary and testis; vitelline reservoir an enlarged duct dorsal to ovary. Uterus preovarian, intercecal, slightly coiled; metraterm straight, inside hermaphroditic sac. Eggs yellowish, 53 to 67 long by 35 to 50 wide (collapsed eggs 48 to 70 by 26 to 42). Excretory pore terminal, excretory vesicle extending to anterior end of testis. Two pairs of well-developed lymph vessels extending length of body and prominent in forebody.

Discussion: Three species of *Hapladena* are known from Tortugas, Florida: *H. varia* Linton, 1910; *H. ovalis* (Linton, 1910) Manter, 1947; and *H. leptotelea* Manter, 1947. The fourth species, *H. acanthuri* Siddiqi & Cable, 1960, is from Puerto Rico.

H. leptotelea is easily identified by the tapered and pointed hindbody and by the longitudinal aperture of the acetabulum. *H. varia* and *H. ovalis* are very similar, but are differentiated by the length of the hermaphroditic sac (extending postacetabular in *H. varia* but not in *H. ovalis*) and the sucker ratio (1: 1 for *H. varia* but 1: 1.34 to 1.5 for *H. ovalis*). Sobolev (1955) also included anterior vitelline extent to distinguish these two species (to the posterior edge of the acetabulum in *H. ovalis* but to mid-acetabulum or farther forward in *H. varia*), but specimens of *H. ovalis* collected by Manter may have vitellaria overlapping the anterior edge of the acetabulum. In length of hermaphroditic sac, *H. acanthuri* resembles *H. ovalis; H. spinosa* resembles *H. varia.*

Both *H. acanthuri* and *H. spinosa* differ from *H. varia* and *H. ovalis* in their more elongate bodies, slightly smaller sucker ratios, and slightly wider eggs. *H. spinosa* differs from *H. acanthuri* chiefly in the sphincter at the genital pore, the unspined metraterm, and the cluster of minute spines at the base of the hermaphroditic duct (resembling *H. ovalis*) which are everted to the surface; in addition, the vitelline follicles are less elongate, the forebody is more tapered, and the seminal receptacle is well developed. *H. spinosa* seems to be more extensively spined than any of the other species.

Hapladena tanyorchis, n. sp.
(Figs. 15–16)

Hosts: Naso sp. (either *N. unicornis* or *N. brevirostris*), kala (Acanthuridae, surgeonfishes); 5 specimens from 1 of 28 hosts. *Naso lituratus* (Bloch and Schneider); 1 specimen from 1 of 2 hosts.

Location: Intestine.

Holotype: U. S. Natl. Mus. Helm. Coll., No. 39474.

Description (based on 4 specimens): Body elongate, 2.848 to 4.154 long by 0.623 to 0.670 wide; posterior end of body rounded, forebody tapered and concave ventrally, anterior end of body rounded; cuticula spinose, spines occurring in some specimens as far posteriorly as mid-testicular level but entirely absent in others. Oral suckers rounded, 0.181 to 0.235 wide by 0.192 to 0.308 long, mouth opening elongate; acetabulum rounded 0.235 to 0.304 wide by 0.221 to 0.375 long, one-eighth to one-sixth body length from anterior end, retractable into body (fig. 15); sucker ratio 1: 1.3. Prepharynx very short; pharynx 0.096 to 0.184 long by 0.080 to 0.128 wide; esophagus 0.201 to 0.272 long; cecal bifurcation dorsal to acetabulum, ceca extending to posterior end of body and often containing blood.

Posttesticular space 0.536 to 0.737. Testis single, median, 0.871 to 1.106 long by 0.154 wide

(0.235 to 0.382 deep), with irregular margin; anterior portion of testis sometimes bent, giving appearance of two tandem, contiguous testes. External seminal vesicle saccular, 0.235 to 0.281 long by 0.114 to 0.147 wide. Hermaphroditic sac 0.335 long by 0.161 wide, dorsal to acetabulum, containing: internal seminal vesicle, 0.201 to 0.268 long by 0.054 to 0.101 wide; inconspicuous prostatic cells; and hermaphroditic duct. Genital pore median, preacetabular, close to acetabulum.

Ovary subglobular, 0.181 to 0.248 long by 0.168 to 0.188 wide, median, 0.174 to 0.408 anterior to testis; Mehlis' gland well developed, immediately preovarian; Laurer's canal not observed; seminal receptacle inconspicuous, 0.136 in diameter in one specimen. Vitelline follicles elongate, often grouped in rosettes, extending from middle or posterior end of external seminal vesicle to posterior end of body, dorsal and lateral to organs of hindbody and meeting ventrally between ovary and testis and posterior to testis. Uterus preovarian, intercecal; metraterm muscular, in hermaphroditic sac. Uncollapsed egg 56 by 37, collapsed eggs 43 to 56 by 24 to 29. Excretory pore terminal, excretory vescicle extending to anterior end of testis. Two pairs of lymphatic vessels extending length of body and ending beside oral sucker. The name *tanyorchis* is from *tany* (=long) and *orchis* (=testis).

One additional specimen was 3.250 long by 0.149 wide, but it was immature (without eggs); another was overly flattened and measured 6.767 by 1.307.

Discussion: This species is like *H. ovalis*, *H. acanthuri*, and *H. spinosa* in the extent of the hermaphroditic sac, but it is distinctive in the elongate mouth opening, the short forebody, the very elongate and irregularly-shaped testis. The elongate vitelline follicles are like those of *H. acanthuri*.

The long, irregularly-shaped testis suggests the genus *Spiritestis* Nagaty, 1948. *Spiritestis* was found to possess an hermaphroditic sac by Montgomery (1957) who transferred it to the family Waretrematidae. In addition, *Spiritestis* has 4 retractile lobes on the oral sucker, a uterine seminal receptacle, and vitellaria restricted to the posterior half of the hindbody.

SUMMARY

The genera of Monorchiidae are grouped in 5 subfamilies. Several genera are discussed briefly with special attention to *Lasiotocus*, *Proctotrema*, *Genolopa*, and *"Pristisomum."*

A new genus, *Chrisomon*, is named for *Telolecithus tropicus* Manter, 1940.

The following new species are named: *Genolopa oculata*, *Monorcheides decapteri*, *Lasiotocus delicatus*, *Hurleytrematoides coronatum*, *Haplodena spinosa*, and *H. tanyorchis*.

The following new combinations are proposed: *Proctotrema chaetodipteri* (Thomas, 1959) (formerly *Lasiotocus*); *P. himezi* (Yam., 1951) (formerly *Lasiotocus*); *P. latum* (Manter, 1942) (formerly *Monorchis*); *Genolopa brevicaecum* (Manter, 1942) (formerly *Paraproctotrema*).

The genus *Pseudohurleytrema* Yam., 1954 is considered a synonym of *Hurleytrema* Srivastava, 1939.

LITERATURE CITED

BRAVO HOLLIS, M. 1956 Tremátodos de peces marinos de aguas mexicanas. XI. Estudio de 17 digeneos de las costas del Pacífico, incluyendo seis especies nuevas y un genero nuevo. An. Inst. Biol., Univ. Nac. Méx. **27**: 245–277.

DOLLFUS, R. PH. 1948 L'enigme de *Distoma furcatum* Bremser enfin expliquée. Contribution à la connaissance des trématodes des poissons du genre *Mullus* en Méditerranée. Bull. Inst. Océanogr. (928) **45**: 23 pp.

LOOSS, A. 1907 Zur Kenntnis der Distomenfamilie Hemiuridae. Zool. Anz. **31**: 585–620.

MANTER, H. W. 1942 Monorchidae (Trematoda) from fishes of Tortugas, Florida. Tr. Am. Micr. Soc. **61**: 349–360.

MARTIN, W. E. 1940 Studies on the trematodes of Woods Hole. III. The life cycle of *Monorcheides cumingiae* (Martin) with special reference to its effect on the invertebrate host. Biol. Bull. **79**: 131–144.

MONTGOMERY, W. R. 1957 Studies on digenetic trematodes from marine fishes of La Jolla, California. Tr. Am. Micr. Soc. **76**: 13–36.

ODHNER, T. 1911 Zum natürlichen System der digenen Trematoden. 2. Zool. Anz. **37**: 237–253.

SOBOLEV, A. A. 1955 Family Monorchidae Odhner, 1911. (*In* Skrjabin, K. I. Trematodes of Animals and Man. Moskva. **11**: 257–464.)

SRIVASTAVA, H. D. 1939 Two new trematodes of the family Monorchidae Odhner, 1911, from Indian marine food fishes. Indian J. Vet. Sci. and An. Husb. **9**: 233–236.

SZIDAT, L. 1950 Los parasitos del rôbalo (*Eleginops maclovinus* Cuv. & Val.) Trab. 1 Cong. Nac. Pesquerias Maritimas (Mar del Plata) **2**: 235–270.

THOMAS, J. D. 1959 Trematodes of Ghanaian sub-littoral fishes. I. The family Monorchiidae. J. Parasit. **45**: 95–113.

TIMON-DAVID, J. 1937 Sur l'evolution experimentale des metacercaires de *Zoogonus mirus* Looss, 1901 (trématodes, famille des Zoogonidae). Ass. Franc. Avance. Sci., C. R. (60. session, Marseille, 1936): 274–276.

VLASENKO, P. V. 1931 Zur Helminthofauna de Schwarzmeerfische. [Russian text; German summary] Trudy Karadag. Biol. Stantsii **4**: 88–136.

YAMAGUTI, S. 1954 Systema Helminthum. Part I. Digenetic Trematodes of Fishes. 405 pp. Tokyo. (Issued Jan. 10)

——— 1958 Systema Helminthum. Volume I. The Digenetic Trematodes of Vertebrates. Parts I and II. 1575 pp. New York, London.

EXPLANATION OF PLATES

All figures drawn (by M.H.P.) with the aid of a camera lucida. The projected scale has its value indicated in millimeters. Abbreviations used: *c*, cirrus; *ce*, cecum; *cs*, cirrus sac; *esv*, external seminal vesicle; *ga*, genital atrium; *gp*, genital pore; *hd*, hermaphroditic duct; *hs*, hermaphroditic sac; *isv*, internal seminal vesicle; *l*, lymph ducts; *lc*, Laurer's canal; *mt*, metraterm; *ov*, ovary; *pc*, prostatic cells; *s*, spines; *sph*, sphincter; *sr*, seminal receptacle; *sv*, seminal vesicle; *t*, testis, *to*, terminal organ; *tov*, terminal organ vesicle; *ut*, uterus; *vr*, vitelline reservoir; *vt*, vitelline follicles.

PLATE I

FIGURE 1. *Genolopa oculata* from *Albula vulpes*, holotype; ventral view.

FIGURE 2. *G. oculata*, paratype; dorsal view of terminal genital ducts.

FIGURE 3. *Monorcheides decapteri* from *Decapterus pinnulatus*, holotype; dorsal view.

FIGURE 4. *M. decapteri*, holotype; ventral view of terminal genital ducts.

FIGURE 5. *Lasiotocus delicatus* from *Naso* sp., holotype; ventral view.

FIGURE 6. *L. delicatus* from *Parupeneus multifasciatus*, paratype; dorsal view of terminal genital ducts.

FIGURE 7. *L. delicatus*, paratype; (a) spines of "metraterm"; (b) spines of cirrus.

PLATE II

FIGURE 8. *Hurleytrematoides coronatum* from *Chaetodon lineolatus*, holotype; ventral view.

FIGURE 9. *H. coronatum*, holotype; ventral view of terminal genital ducts.

FIGURE 10. *H. coronatum from Chaetodon fremblii*, paratype, portion of ceca of a shorter specimen.

FIGURE 11. *H. coronatum*, eggs from uterus of squashed paratype from *Chaetodon lineolatus*.

FIGURE 12. *Hapladena spinosa* from *Acanthurus sandvicensis*, holotype; ventro-lateral view.

FIGURE 13. *H. spinosa*, paratype; ventral view of terminal genital ducts with hermaphroditic duct not everted and spines located in lumen immediately anterior to entrance of seminal vesicle.

FIGURE 14. *H. spinosa*, holotype; ventral view of terminal genital ducts with hermaphroditic duct everted, bringing spines to surface.

FIGURE 15. *Hapladena tanyorchis* from *Naso* sp., holotype; ventral view.

FIGURE 16. *H. tanyorchis* from *Naso lituratus*, paratype; ventral view of anterior part of body (pharynx in lumen of oral sucker).

PLATE I

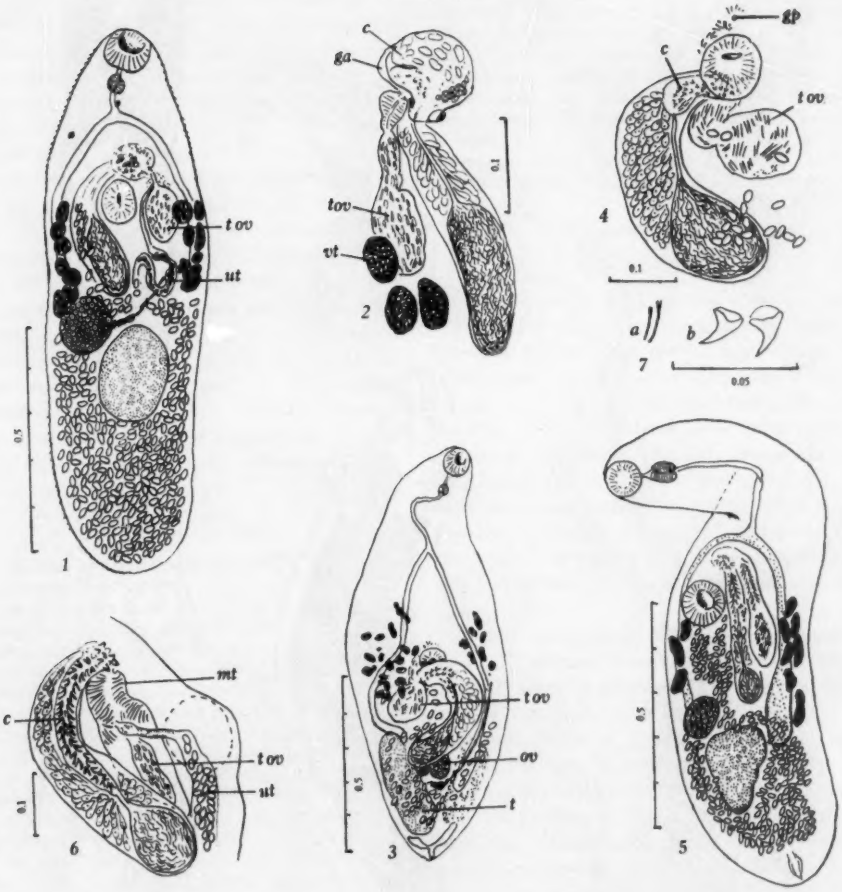
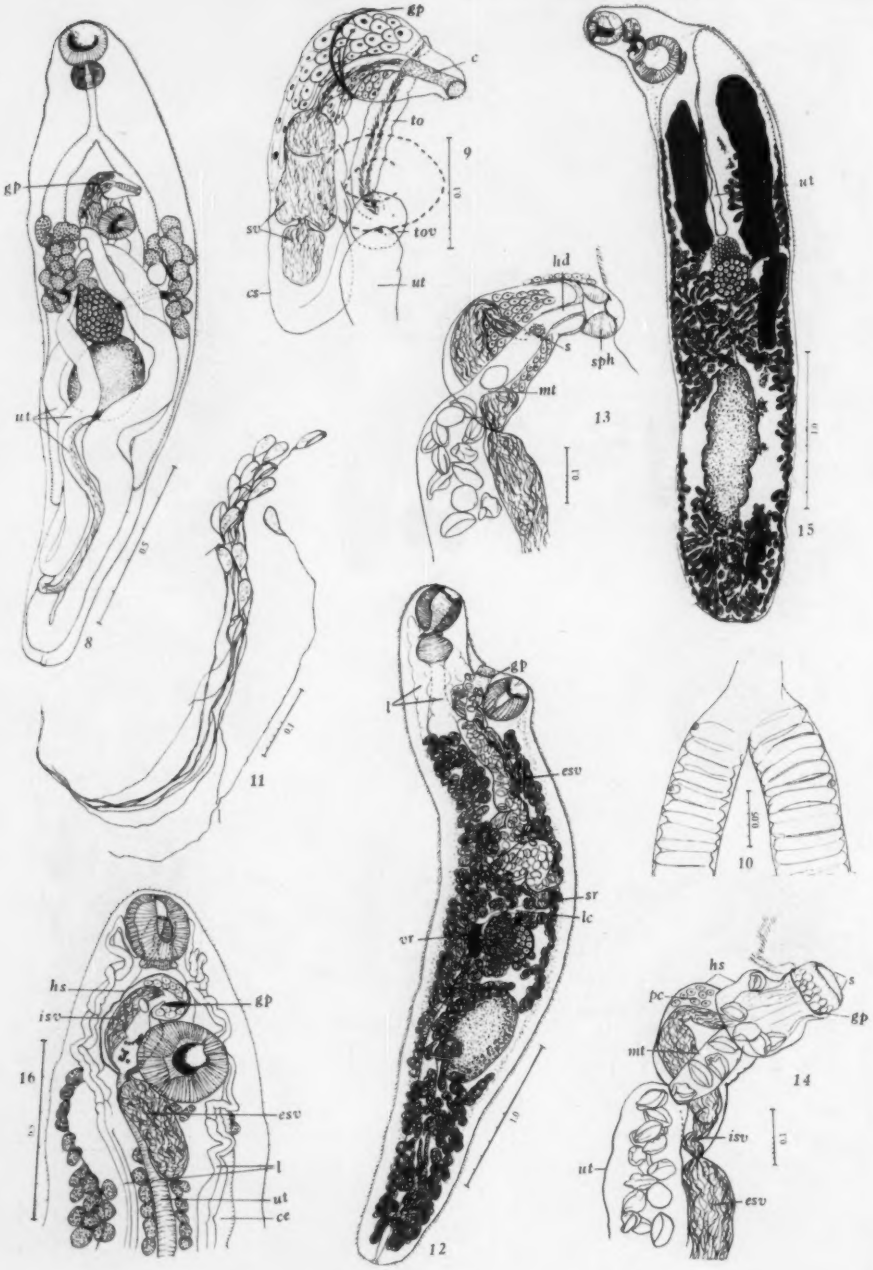

t ov
ut
0.5
1
c
ga
0.1
tov
vt
2
gp
c
t ov
4
0.1
a
b
7
0.05
mt
c
t ov
ut
0.1
6
t ov
ov
t
0.5
3
0.5
5

PLATE II


gp
ut
0.5
8
gp
c
to
9
0.1
su
tov
cs
ut
hd
s
sph
mt
13
0.1
ut
1.0
15
11
0.1
gp
l
esv
sr
lc
vr
1.0
12
10
0.05
hs
isv
gp
16
0.5
esv
l
ut
ce
hs
pc
s
gp
mt
14
isv
0.1
ut
esv

DEVELOPMENT OF MOTHER AND DAUGHTER SPOROCYSTS OF *HAPLOMETRANA INTESTINALIS* LUCKER, A PLAGIORCHIOID TREMATODE OF FROGS*

STEWART C. SCHELL

Department of Biological Sciences, University of Idaho, Moscow

Haplometrana intestinalis was originally described by Lucker in 1931 from *Rana pretiosa* in Washington State. In 1937, Olsen described *H. utahensis* as a second species in the genus and also reported its life history. Olsen's specimens were collected in Washington, Utah. Waitz (1959) carefully examined numerous specimens collected in Idaho as well as specimens sent to him by Drs. Lucker and Olsen and concluded that *H. intestinalis* and *H. utahensis* were conspecific. Waitz accordingly declared *H. utahensis* as a synonym of *H. intestinalis*. In reporting the life history, Olsen provided an illustration and a description of a 27-day-old daughter sporocyst. The mother sporocyst and early developmental stages of the daughter sporocyst were not included. The work reported here is aimed at learning as much as possible about the development of the mother and daughter sporocysts.

METHODS AND MATERIALS

Laboratory reared specimens of *Physa ampullacea* Gould and *P. gyrina* Say were used throughout the investigation. Mature snails of these species were collected during May and June and placed in aquaria for oviposition. As egg masses were released they were removed and placed in large fingerbowls of water for hatching. As the snails hatched, dried maple leaves and powdered calcium carbonate were added as food. Young snails were infected by placing 20 to 30 specimens in stender dishes containing embryonated eggs of *H. intestinalis*. The snails were allowed to ingest eggs for about 1 hour after which they were transferred to large fingerbowls in which they were maintained as described above. Laboratory temperatures ranged from 22 to 30 C during the maintenance period.

Most observations were made from living specimens. Intra vitam staining with dilute neutral red or Nile blue was useful for demonstration of germinal and soma cells in the developing sporocysts and also for staining the host cell nuclei. Living material was studied with the aid of a compound binocular microscope equipped with 15× oculars and fluorite oil immersion objectives. Infected snails were dissected in a 0.2 percent sodium chloride solution. Some infected snails were fixed in Gilson's fluid for later sectioning and staining. Permanent stains used for sections were Ehrlich's haematoxylin and Heidenhain's iron-haematoxylin.

OBSERVATIONS

The Miracidium

Hatching occurs in the snail stomach within 1 hour after eggs are ingested. Just prior to hatching, the miracidia become quite active. The entire body undergoes repeated contractions, sometimes as a peristaltic wave that passes over the body from end to end while at other times movement consists of alternate shortening and lengthening of the body. The miracidia are weak swimmers, usually swimming rather slowly for short distances, then stopping to rest on the glass slide. They tend to adhere by their anterior end to bits of debris or to pieces of host tissue.

The miracidium (fig. 2) has a body length of 38 to 45 microns when fully extended and a width of 12 to 14 microns. The body wall is extremely thin and transparent, without sign of epidermal plates. A spherical bulb with longitudinal striations is located in the anterior end of the body. Bulb dimensions are 8 microns long by 6 microns wide. The function of the bulb was not determined. Two large nucleated penetration glands occupy about two-thirds of the body of the miracidium. The glands contain a secretion which causes them to have a coarsely granular appearance. The granules stain brilliantly with neutral red. The glands differ in size, one being twice as long as the other. It was noticed that after the miracidia penetrated the snail digestive tract the glands became almost invisible, losing their granular appear-

Received for publication December 13, 1960.

* This investigation was supported by a research grant (#E-950) from the National Institutes of Health, U. S. Public Health Service.

ance. It is possible that the gland secretion is used during penetration of the epithelium. Two nuclei are associated with each penetration gland. In the anterior half of the miracidium there is also a pair of small spindle-shaped glands (figs. 1 and 2). Each gland has a long curved duct leading forward to the anterior end of the bulb. These glands and their ducts contain a granular secretion similar to that of the large penetration glands just described. It appears that these glands function during hatching or shortly thereafter, as they are more plainly visible through the egg shell before hatching than they are after hatching.

A pair of flame cells is located in the posterior part of the miracidium. In this same area two germinal cells can be seen, each having a nucleus with a large endosome. The cytoplasm and endosome stain a deep red with neutral red. These cells are of the same size, have the same structure, and exhibit the same staining characteristics as the germinal cells produced later in the young mother sporocyst. The number and location of the germinal cells is always the same. In addition to the germinal cells, the nuclei of two other cells can be seen. One of these is always located between the germinal cells, while the other is off to one side of the body. They may be the nuclei of soma cells. Cilia cover the entire surface of the miracidium, long ones on the anterior part of the body and progressively shorter ones on the posterior part. The ciliary beat is rather weak, frequently failing to be synchronized.

Following hatching, the miracidium penetrates the epithelium of either the stomach, cecum or fore part of the intestine. Penetration appears to be due largely to the action of cytolytic secretions. Miracidia observed in the act of penetrating appear to undergo very little movement. The epithelia cells adjacent to the miracidium become quite clear and amorphous. The miracidia penetrate only the epithelium of the snail digestive tract, finally lodging between the base of the epithelium and the underlying basement membrane. The original site of penetration can be detected for some time as a minute break or slight derangement of the epithelium cells.

Considerable confusion prevails regarding the anatomy of the plagiorchioid miracidium. Krull (1931) illustrates the miracidium of *Pneumonoeces medioplexus* Stafford as having a longitudinally striated bulb, a gut with four nuclei, distinct epidermal plates, a pair of flame cells and two germinal cells at the posterior end. Olsen (1937) illustrated the miracidium of *H. utahensis* as having a digestive system, four or five germ balls and two small penetration glands. Flame cells were not shown. The germ balls illustrated by Olsen were probably the nuclei of the large penetration glands mentioned in the present account of *H. intestinalis*. The penetration glands shown by Olsen are most likely the same as the spindle-shaped glands observed by the writer. The only author who illustrated and described the large penetration glands is Talbot (1933). He shows two large granular glands in the miracidium of *Lechriorchis primus* Stafford. He illustrated germ cells. He also included a primitive gut. Walker (1939) studied the miracidium of *Renifer aniarum* (Leidy) which he illustrated as having a papilla (= bulb), an elementary digestive organ, a pair of secretory glands, one large germ cell in the middle of the body, and two flame cells at the posterior end. The papilla was protrusible.

It is obvious that additional detailed studies of plagiorchioid miracidia are needed. To date, the writer has been able to examine the miracidia of six species of plagiorchioid trematodes. The structural variations between species appear to be exceedingly minute. A more complete report will be presented on this subject at a later date.

The Mother and Daughter Sporocysts

After penetrating the epithelium of the snail digestive tract, the miracidium quickly metamorphoses to the mother sporocyst stage through the loss of cilia and the bulb. The large penetration glands can no longer be seen, but their nuclei remain visible for about 24 hours longer. The young mother sporocyst is about 30 percent smaller than the miracidium. This decrease in size could be due to the loss of secretion from the penetration glands, or it could be due to water loss. A young mother sporocyst 18 hours old is shown in figure 3. It is lodged between the epithelium and the basement membrane. In it the two germinal cells lie at one end. The four gland cell nuclei and two soma cell nuclei are also visible, as are the flame cells. This specimen was 21 microns long by 14 microns wide. The parasite causes the base of the

epithelium to be indented while the basement membrane bulges above the outer surface of the intestinal wall. This specimen was located in the intestinal wall just a short distance behind the cecum.

By the second day the mother sporocyst (fig. 4) has undergone only a slight increase in size. Germinal and soma cells have multiplied. Flame cells are still visible in some specimens. The cells in the basement membrane of the host intestine have undergone multiplication to the extent that they now form a layer between the parasite and the epithelium. The parasite is now encysted.

By the end of the third day the encysted mother sporocyst (fig. 5) assumes a more spherical shape. Counts made on germinal cells in several mother sporocysts of this age ranged from 12 to 19. The sporocysts had attained a diameter of 30 to 35 microns. The limiting membrane of the mother sporocyst is still visible, especially when the sporocyst undergoes contractions. In older stages the limiting membrane is so closely applied to the inner surface of the cyst membrane that the former could no longer be detected. After the third day development progresses more rapidly. A 4-day-old mother sporocyst had dimensions of 51 microns by 29 microns and contained at least 40 germinal cells.

A 6-day-old specimen is illustrated in figure 6. The cyst protrudes from the wall of the intestine for a distance of 56 microns. The transverse diameter is 120 microns. The sporocyst contains numerous germinal and soma cells. All of the encysted mother sporocysts are at this time colorless and transparent, appearing as clear hemispherical blisters or bubbles attached to the outer surface of the snail digestive tract.

Multiplication of germinal cells continues until the ninth day, when daughter sporocyst embryos and small clusters of germinal cells start to form. By the 10th day (fig. 7) a considerable number of daughter sporocyst embryos are present. The mother sporocysts are now spherical, having a diameter of 266 to 290 microns. Growth causes the cyst to become pedunculate. Individual daughter sporocyst embryos measured 37 to 45 microns by 30 to 37 microns. Numerous germinal cells and germ balls are dispersed among the embryos.

Daughter sporocyst development is well underway by the 16th day, each sporocyst acquiring a characteristic shape (fig. 8) by developing knob-like growth at one or both ends. Under high magnification it could be seen that cercarial embryo production had already begun in these young daughter sporocysts. In addition to the daughter sporocysts, some individual germinal cells and germ balls are also present at this time. The bumpy or uneven surface of the cyst is shown in figure 8. This is thought to be due jointly to the growth of the daughter sporocysts, pressing against the inner surface of the cyst membrane, and to the invasion of the mother sporocyst by connective tissue cells of the cyst membrane. This invasion by connective tissue finally results in the development of membranous partitions between the developing daughter sporocysts. This became more evident by the 18th day, at which time each daughter sporocyst had its own enclosing membrane. A somewhat flattened 18-day-old specimen is shown in figure 9. In such specimens the invasion by host connective tissue is plainly evident. By this time germinal cells and germ balls are no longer present. It appears as if daughter sporocyst production is concluded.

The 21-day-old daughter sporocyst had become differentiated to the extent that a distinct body wall and a body cavity filled with cercarial embryos was present. The entire mass of daughter sporocysts (fig. 11) is held together by the original cyst membrane. A single daughter sporocyst dissected from the mass is illustrated in figure 12. The membrane enclosing it was formed from host connective tissue cells. Observation under high magnification revealed that the membrane had now assumed the appearance of an epithelium, composed of very clear flat or oval epithelial cells. It was noticed that the appearance of the epithelial membrane depended largely upon the condition of the daughter sporocyst enclosed by it. If the sporocyst was in a normal relaxed state, the membrane cells appeared flat. If the sporocyst was partially contracted, the membrane cells tended to be cuboidal or rounded. With strong contractions, especially at one end or the other, the sporocyst body wall was drawn away from the membrane, leaving a space between the two. This indicates clearly that the membrane is not attached to the daughter sporocysts but merely encloses them.

Figure 13 is a drawing of a portion of a stained section from an infected snail fixed on the 24th day. It reveals details of structure that

could not be seen in entire daughter sporocyst masses. The host connective tissue membranes are visible between the daughter sporocysts. In some daughter sporocysts mitotic figures of dividing germinal cells are visible. Germinal and soma cell nuclei could be identified easily in the cercarial embryos.

The first cercariae were found in several daughter sporocysts on the 27th day. Usually these sporocysts contained a single cercaria that seemed to be fully developed except for the finfold on the tail. Enclosed with the cercaria were several cercarial embryos and the germinal mass. Cercariae began to emerge from laboratory infected snails on the 34th day. A 35-day-old daughter sporocyst is shown in figure 10. Cercariae in all stages of development, germ balls, and germinal cells are present. The sporocyst is attached to others in the sporocyst mass, the entire mass being held together by the original cyst membrane. Daughter sporocysts were never found free in the snail. In its fully developed final stages, the sporocyst mass may attain a diameter of 1.5 to 2.5 mm. The masses press against the snail digestive gland, crowding it to one side, and creating the false impression of invading the gland. The masses might eventually become loosely attached to the digestive gland by strands of connective tissue, but the gland tissue is never invaded or infiltrated directly by the daughter sporocysts.

Although a birth pore was not observed, cercariae were seen escaping from one end of the daughter sporocysts. In a few cases cercariae were found between the daughter sporocyst and the enclosing membrane. It is not known how cercariae break out of this membrane.

Olsen (1937), in describing the cercaria of *H. utahensis*, mentioned that it had a dorsal finfold. The cercariae observed in this investigation had a ventral finfold. The cercaria belongs to the Ornatae group of xiphidiocercariae. With its club-shaped excretory bladder it exhibits a relationship with the cercaria of *Alloglossidium corti* (Lamont) and *Macroderoides typicus* (Winfield), both of which were assigned to the family Macroderoididae by McMullen (1937). Cercariae in this family have a club-shaped excretory bladder, intestinal ceca that extend to the posterior end of the body, and a finfold on the tail.

DISCUSSION

In reporting plagiorchioid life history studies a number of authors have mentioned the paletot which exists as a cellular coat enclosing the daughter sporocysts. The writer believes that the membrane which ultimately encloses the daughter sporocysts of *H. intestinalis* is a paletot. It is emphasized that this structure originated from the basement membrane cells of the host snail. The development of a cyst membrane around the mother sporocyst and later the development of membranes around individual daughter sporocysts are interpreted as cellular responses on the part of the host. As a result of this, the larval parasite never invades the body of the host snail beyond the gut epithelium. As the daughter sporocyst mass enlarges, it tends to press against the digestive gland, but the gland tissue is never actually invaded by the daughter sporocysts.

Cort and Ameel (1944) studied the sporocyst development of a number of species of Plagiorchioidea in naturally infected snails. Among the species studied were *Alloglossidium corti* and *Macroderoides typicus*, which as indicated previously were assigned to the family Macroderoididae by McMullen (1937). Since *Haplometrana intestinalis* also belongs in this family, on the basis of cercarial anatomy, the findings of the above authors are of direct interest. They found that all daughter sporocysts of *A. corti*, developing from one mother sporocyst, were concentrated in one mass (as in *H. intestinalis*) and were attached to each other by their paletot cells. Daughter sporocysts were entirely separated from the tissue of the digestive gland. The daughter sporocysts of *M. typicus* on the other hand developed in a linear series in long unbranched tubules which in turn were formed by the paletot cells. These authors maintained that its paletot cells originated from the mother sporocyst. Due to the fact that the work of Cort and Ameel was done with naturally infected snails and the early stages of mother sporocyst development were not observed, it is highly desirable that a detailed study be made of sporocyst development of *A. corti* and *M. typicus*, using laboratory-reared snails. Special attention should be paid to the development of the mother sporocyst. Cort, Ameel and Van der Woude (1952) studied the sporocyst development of *Lechriorchis*

primus Stafford, paying attention to the origin and development of the paletot. Laboratory reared snails were used in this investigation. They maintained that the paletot was formed from cells that separated from the daughter sporocyst embryos. Sporocyst development of *L. primus* differed in some respects from that of *H. intestinalis* in that the mother sporocyst underwent profuse branching and daughter sporocysts were released from the mother sporocyst and migrated into the surrounding snail tissues. The migrating daughter sporocysts were each enclosed in a paletot. The findings regarding paletot origin and development in *L. primus, A. corti,* and *M. typicus* obviously do not agree with what is reported here for *H. intestinalis*. With so little known about plagiorchioid larval development at the present time, definite statements cannot be made regarding the existing descrepancy. The writer is investigating the sporocyst development of several other species in the superfamily Plagiorchiodea. After these studies are completed, it might be possible to draw conclusions regarding sporocyst development and the origin of paletot cells.

SUMMARY

Development of the mother and daughter sporocysts of *Haplometrana intestinalis* was studied in laboratory reared specimens of *Physa gyrina* and *P. ampullacea*. The miracidium hatches in the snail stomach and then penetrates the epithelium of the snail digestive tract, finally lodging between the epithelium and the underlying basement membrane. Here the miracidium metamorphoses to the mother sporocyst stage. The developing mother sporocysts are restricted to the wall of the stomach, cecum, and fore part of the intestine. As the mother sporocyst develops, basement membrane cells form a cyst membrane around it. Multiplication of germinal cells continues in the mother sporocyst up to the ninth day at which time daughter sporocyst embryos start to appear.

Production of daughter sporocyst embryos ceased by the 18th day. Cells of the cyst membrane invade the mother sporocyst, creating separate cellular envelopes around each daughter sporocyst. These envelopes, formed by host tissue cells, are the paletots. Paletot formation is the result of a host cellular response to the invading parasite. The daughter sporocysts are held together in a single mass by the paletot cells. Daughter sporocysts never invade snail tissues.

Natural emergence of cercariae began on the 34th day. On the basis of cercarial anatomy, *H. intestinalis* is assigned to the plagiorchioid family Macroderoididae. The cercariae belong to the Ornatae group of xiphidiocercariae which have club-shaped excretory bladder, intestinal ceca that extend to the posterior end of the body, and finfold on the tail.

REFERENCES

CORT, W. W. AND AMEEL, D. J. 1944 Further studies on the development of the sporocyst stages of plagiorchiid trematodes. J. Parasit. **30**: 37–56.

CORT, W. W.; AMEEL, D. J. AND VAN DER WOUDE, A. 1952 Development of the mother and daughter sporocysts of a snake plagiorchioid, *Lechriorchis primus* (Trematoda: Reniferidae). J. Parasit. **38**: 187–202.

KRULL, W. H. 1931 Life history studies on two frog lung flukes, *Pneumonoeces medioplexus* and *Pneumobites parviplexus*. Tr. Am. Micr. Soc. **50**: 215–277.

LUCKER, J. T. 1931 A new genus and new species of trematode worms of the family Plagiorchiidae. Proc. U. S. Natl. Mus. **79**: 1–8.

MCMULLEN, D. B. 1937 A discussion of the taxonomy of the family Plagiorchiidae Lühe, 1901, and related trematodes. J. Parasit. **23**: 244–258.

OLSEN, O. W. 1937 Description and life history of the trematode *Haplometrana utahensis* sp. nov. (Plagiorchiidae) from *Rana pretiosa*. J. Parasit. **23**: 13-28.

TALBOT, S. B. 1933 Life history studies on trematodes of the subfamily Reniferinae. Parasitology **25**: 518–545.

WAITZ, J. A. 1959 A revision of the genus *Haplometrana* Lucker, 1931 (Trematoda: Plagiorchiidae) with notes on its distribution and specificity. J. Parasit. **45**: 385–388.

WALKER, J. H. 1939 Experimental studies on trematodes belonging to the subfamily Reniferinae. Tr. Am. Micr. Soc. **58**: 404–430.

Explanation of Plates
Development of *Haplometrana intestinalis*

Plate I

1, embryonated egg; 2, miracidium; 3, very young mother sporocyst, lodged between epithelium and basement membrane, 18 hours old; 4, young mother sporocyst, 2 days old, encysted; 5, young mother sporocyst, 3 days old; 6, mother sporocyst, filled with germinal cells, 6 days old; 7, pedunculate mother sporocyst, 10 days old, containing daughter sporocyst embryos, germinal cells and germ balls; 8, mother sporocyst, 16 days old, surface becoming uneven.

Plate II

9, mother sporocyst, 18 days old, showing invasion by connective tissue cells; 10, daughter sporocyst, 35 days old; 11, mass of daughter sporocysts attached to snail stomach wall; 12, daughter sporocyst dissected from mass; 13, section of a 24-day-old daughter sporocyst mass, showing membranes around daughter sporocysts; 14, several mother sporocysts attached to wall of stomach, cecum and intestine of snail.

ANNOUNCEMENT

INFORMATION CONCERNING THE JOURNAL OF PARASITOLOGY.

Members of the Society and other readers of the Journal are informed that the "backlog" of manuscripts awaiting publication in the Journal of Parasitology no longer exists, for every galley on hand this date (May 9, 1961) will appear in the June 1961 issue. There are necessarily, however, a considerable number of manuscripts in process of being reviewed or of revision by authors, or awaiting setting into type at the Press. Publication of manuscripts acceptable for publication within 6 or 7 months after date of receipt in the Editor's office is now feasible providing, of course, there are no interruptions in the routine of processing, such as inordinate delays in returning manuscripts on the part of authors or reviewers. Reviewers and the Press are cooperating closely with the editorial office in expediting publication.

The Editorial Board and past and present officers of the Society and members of Council are fairly well agreed with the proposal that it would be desirable for the Journal to start publishing general papers, such as reviews or treatises on particular subjects within the field of parasitology. To this end the Editor has appointed the following committee of assistant editors to assist him: Drs. Kenneth C. Kates, R. Barclay McGhee, and Paul P. Weinstein. While these general papers will be "invited," an investigator with an appropriate contribution in mind can properly make the fact known to any member of the above committee, the Editor, or one of the Associate Editors, who together decide whether an invitation will be extended. Suggestions and ideas are welcome!

Also, the publication of book reviews has been given serious consideration. About half of the members consulted favored it, but about half expressed objections. There were three main objections to instituting them: (1) Certain other journals already provide adequate coverage of new books; (2) the space could be used for publication of original work; (3) the service might be misused in various ways. The following proposal that has been adopted represents a compromise: (1) Book reviews will cover only publications entirely or predominantly concerned with parasitology; (2) reviews will be brief and to the point, and will ordinarily not exceed 200 words.

One of the proposals expressed by Dr. Harold W. Brown in a letter circularized during his term as president of the Society, viz, that the scope of the Journal be expanded, has met with general approval. Manuscripts in any area reporting original work dealing with the nature of the parasite or with the parasitic relation will henceforth be considered for publication.—E. R. Becker, *Editor.*

PLATE I

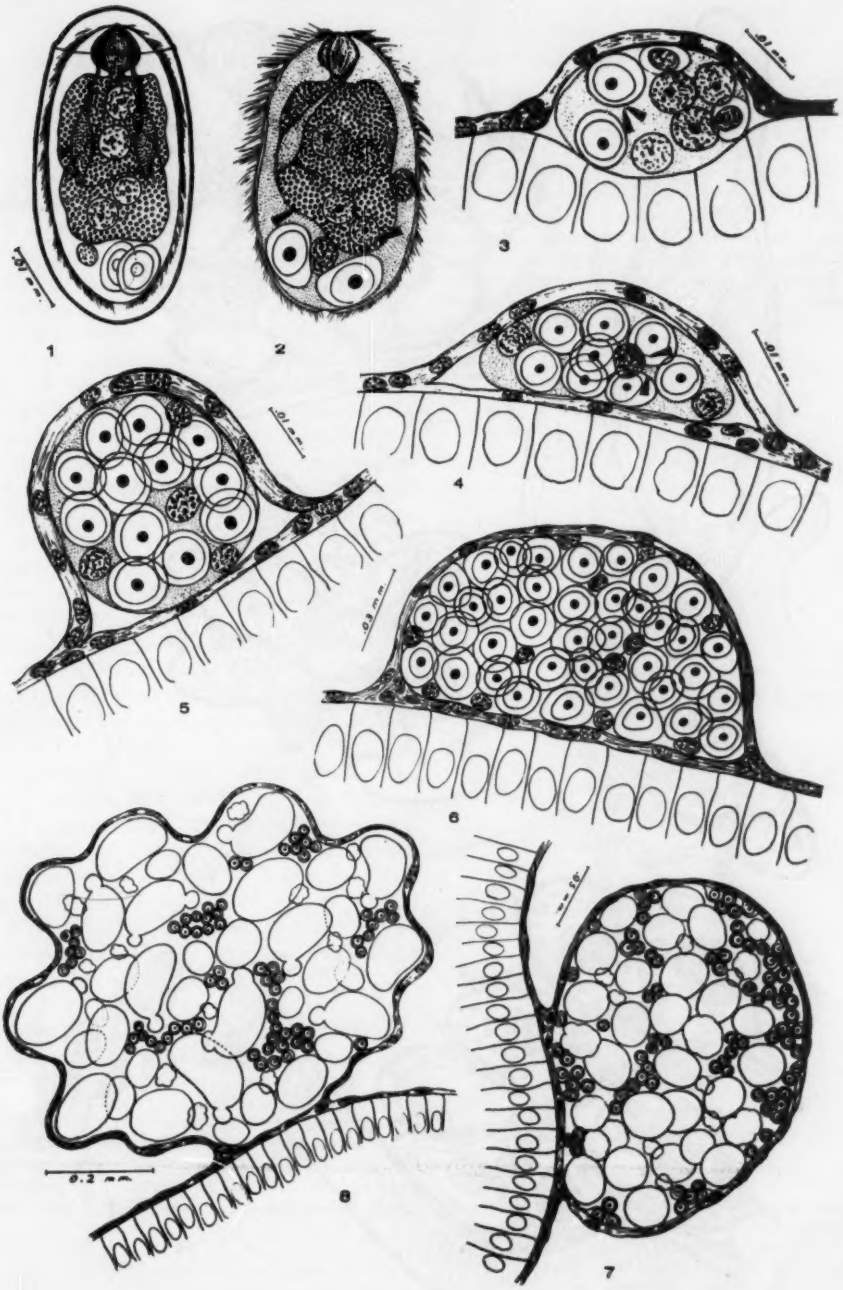
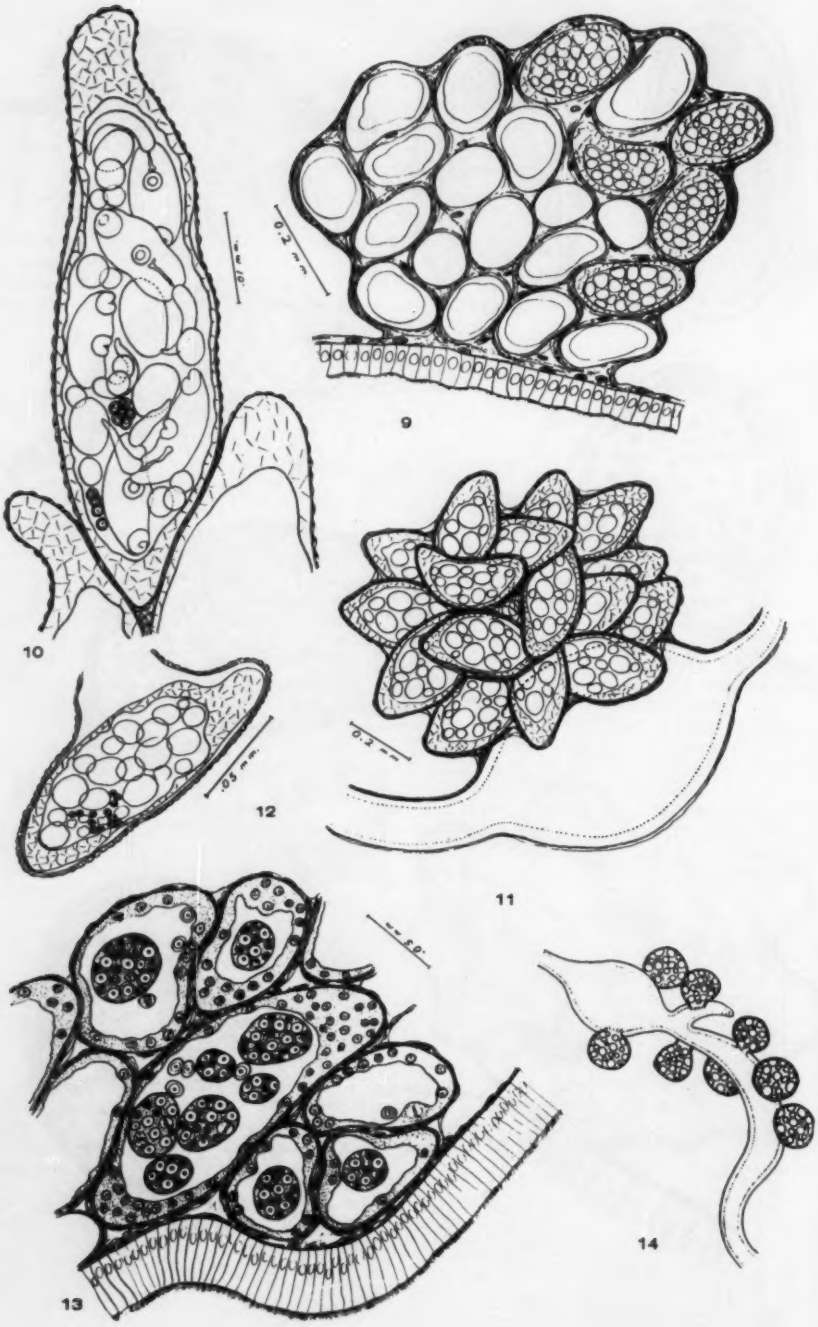


PLATE II



A NEW TREMATODE, *OPISTHIOGLYPHE ORIENTALIS* N. SP. (PLAGIORCHIIDAE), FROM THE GIANT SALAMANDER IN CHINA

HAN JONG RIM* AND F. G. WALLACE

Department of Zoology, University of Minnesota, Minneapolis 14

An unknown species of the genus *Opisthioglyphe* was collected by the junior author in 1936 from the intestine of a giant salamander (*Megalobatrachus japonicus* Temminck) purchased in Canton, China. The locality of origin is unknown, because these salamanders are brought to market from remote areas. Two species of the genus have been found in insectivores (*O. locellus* Kossack, 1910, and *O. megastomus* Baer, 1944) and three in reptiles (*O. magnus* Szidat, 1932, *O. adulescens* Nicoll, 1914, and *O. pelusios* Dollfus, 1950). Four species have been recorded from amphibians: *O. ranae* (Froelich, 1791) Looss, 1907 from *Rana esculenta, R. temporaria, Bufo vulgaris,* and *Molge cristata; O. siredonis* (Poirier, 1886) from *Siredon mexicanus* [*Ambystoma tigrinum*]; *O. hystrix* (Molin, 1861) from *Rana esculenta, R. arvalis, R. temporaria,* and *Bufo vulgaris;* and *O. rastellus* (Olsson, 1876) from *R. temporaria.* According to Yamaguti (1958), *O. xenopi* Porter (1938) from the mantle of *Lymnaea natalensis* may be the larva of *O. ranae.* Reviews of the genus have been given by Perkins (1928), Travassos (1930), Baer (1944), Dollfus (1950), and Yamaguti (1958). *O. hystrix* is redescribed and figured by Perkins (1928) and *O. locellus* is redescribed and figured by Macey (sic) and Moore (1958).

The following description is based on six specimens stained with borax carmine. Measurements are in millimeters.

Opisthioglyphe orientalis n. sp. (Fig. 1)

Description: Body elongate with slightly tapering extremities, three times as long as wide, widest at middle, 1.75 (1.65–1.92) long by 0.50 (0.45 to 0.54) greatest breadth; cuticle minutely spined in anterior half of body. Oral sucker 0.170 long by 0.174 wide; acetabulum 0.170 long by 0.220 wide, at a distance of 0.377 (0.294 to 0.474) from anterior end of body. Pharynx 0.088 × 0.087 with very short prepharynx; oesophagus short; bifurcation of intestine approximately midway between suckers or near ventral sucker; ceca terminating in posterior one-eighth of body. Genital aperture immediately posterior to intestinal bifurcation; cirrus pouch smaller than diameter of ventral sucker, pyriform, slightly overlapping anterior border of ventral sucker; seminal vesicle in proximal half of cirrus pouch. Testes irregular, almost contiguous, intercecal, in tandem or slightly oblique; anterior testis 0.55 × 0.170, somewhat posterior to mid-level of body; posterior testis 0.171 × 0.206, partly within last third of body. Ovary ovoid, 0.121 × 0.134, near right posterior border of acetabulum and touching right cecum;

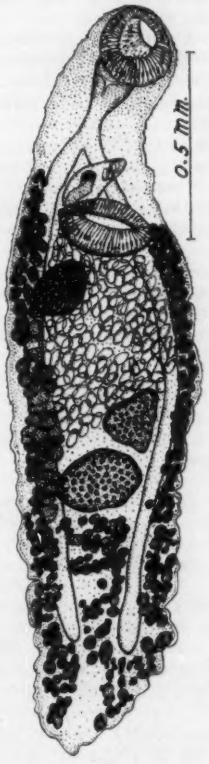


FIGURE 1. *Ospisthioglyphe orientalis,* ventral view.

Received for publication September 19, 1960.

* Department of Parasitology, College of Medicine, Seoul National University, Seoul, Korea.

Mehlis' gland to left of ovary. Vitellaria well developed with follicles in lateral fields, overlapping ceca posterior to level of intestinal bifurcation and filling post-testicular space. Uterus between testes and intestinal bifurcation, not extending beyond ceca. Eggs yellowish-brown, 0.041 × 0.027.

Host: *Megalobatrachus japonicus* Temminck.
Location: Intestine.
Locality: China.
Type: U.S.N.M. Helm. Coll. No. 39053.

The differential characters of the species of the genus *Opisthioglyphe* are brought out in the following key:

1. Testes tandem, uterus entirely anterior to first testis 2
   Testes slightly oblique, uterus not entirely anterior to first testis 4
2. Cirrus pouch extremely large, curving around acetabulum ..... *O. locellus*
   Cirrus pouch not large, pyriform, confined to space between the acetabulum and intestinal bifurcation 3
3. Ceca terminating 1/8 body length from posterior tip ..... *O. pelusios*
   Ceca terminating 2/3 body length from posterior tip ..... *O. adulescens*
4. Body length more than 5 mm 5
   Body length less than 5 mm 6
5. Vitellaria not reaching level of oral sucker, not confluent posterior to testes ..... *O. hystrix*
   Vitellaria extending from posterior edge of oral sucker to posterior end of body, confluent posterior to testes ..... *O. magnus*
6. Oral sucker larger than ventral sucker 7
   Oral sucker equal to or smaller than ventral. *O. orientalis*
7. Esophagus longer than pharynx 8
   Esophagus shorter than pharynx 9
8. Eggs 0.046 × 0.038 mm ..... *O. ranae*
   Eggs 0.06 × 0.03 mm ..... *O. siredonis*
9. Uterus extending laterally beyond ceca. Oral sucker little larger than ventral sucker. *O. rastellus*
   Uterus entirely between ceca. Oral sucker more than twice diameter of ventral sucker. *O. megastomus*

References Cited

Baer, J. G. 1944 Les trematodes parasites de la musaraigne d'eau, *Neomys fodiens* (Schreb.). Bull. Soc. Neuchâtel. Sci. Nat. **68**: 33–84.

Dollfus, R. Ph. 1950 Trematodes récoltes au Congo Belge par le Prof. Paul Brien (mai-aout 1937). Ann. Mus. du Congo Belge C, Zool. Ser. 5, 1 Fasc. **1**: 1–136.

Macey, Ralph W. and Donald J. Moore 1958 The Life Cycle of *Opisthioglyphe locellus* Kossack, 1910, With a Redescription of the Species. Trans. Am. Micr. Soc. **77**: 396–403.

Perkins, M. 1928 A review of the Telorchiinae, a group of distomatid trematodes. Parasit. **20**: 336–356.

Travassos, L. 1930 Pesquizas helminthologicos realizadas em Hamburgo. IV. Notas sobre o genero Opisthioglyphe Looss, 1899, e generos proximos. Mem. Inst. Osw. Cruz **24**: 1–17.

Yamaguti, S. 1958 Systema Helminthum. Vol. I. The Digenetic Trematodes of Vertebrates. Interscience Publishers, New York.

MACRAVESTIBULUM KRAATZI N. SP. (TREMATODA: PRONOCEPHALIDAE), WITH REVISION OF THE GENUS *MACRAVESTIBULUM* MACKIN, 1930

RAYMOND T. DAMIAN
Florida State University, Tallahassee

In March 1959, two monostome flukes were discovered in the intestine of a Florida terrapin, *Pseudemys floridana* (Le Conte), taken from Natural Bridge Sink, part of the St. Mark's River in the Florida panhandle. In June 1960, an additional specimen was recovered from the same host species from the same locality. These trematodes appear to represent a new species in the genus *Macravestibulum* Mackin, 1930, and are herein described as *Macravestibulum kraatzi* n. sp. in honor of Dr. Walter C. Kraatz, Professor Emeritus, Biology Department of The University of Akron.

The genus *Macravestibulum* was erected by Mackin (1930) for a new species, *M. obtusicaudum*, parasitizing the turtle *Pseudemys elegans* from Oklahoma. The genus was assigned to the family Pronocephalidae Looss, 1902. Horsfall (1930) described *Cercaria infracaudata* and later (1935), on the basis of life history studies, regarded it as a larval stage of *M. obtusicaudum*. Hsü (1937) reported the morphology and life-cycle of the second species in the genus, *M. eversum*, from *Graptemys geographica* in Michigan. This was followed by the description of a third species, *M. kepneri* Jones, Mounts and Wolcott, 1945, from *Pseudemys troostii* and *P. texana* in Texas.

In addition to the description of *Macravestibulum kraatzi* n. sp., the present paper includes additions to the descriptions of the other three species in the genus; a revision of the genus *Macravestibulum* Mackin, 1930; and a discussion of the subfamily assignment of *Macravestibulum*. A brief report on the present species has recently appeared in abstract (Damian, 1960).

METHODS

The worms were fixed in A.F.A. (alcohol-formol-acetic) solution and stained with Semichon's aceto-carmine. After being studied as whole mounts, two were removed from their mountings, sectioned frontally at 10 and 15 microns, respectively, and restained with Ehrlich's acid haematoxylin and eosin. Drawings were made with the aid of a micro-projector and camera lucida. All measurements are given in millimeters and indicate extremes of the three specimens.

Received for publication September 22, 1960.

Macravestibulum kraatzi n. sp.
(Figs. 1–5)

Description: With characters of genus *Macravestibulum* revised (see below). Small, thick flukes, length 2.29 to 3.00, width 0.54 to 0.63 at level of vitellaria. Oral sucker 0.105 to 0.145 by 0.098 to 0.105, esophagus 0.23 to 0.37. Ceca simple, becoming slightly sinuous posteriorly, ending at level near posterior border of right (posterior) testis.

Lateral canals of excretory bladder joining anteriorly and ventral to esophagus to form a short median canal which extends to posterior border of oral sucker; vestibular cavity (secondary bladder) lacking large conspicuous diverticula from lateral chambers.

Testes subspherical to ovoid, left one 0.175 to 0.335 by 0.165 to 0.270; right testis 0.208 to 0.302 by 0.165 to 0.227. Vasa efferentia leaving testes laterally, curving outside of ceca, then curving medially to unite just anterior to ovary, forming vas deferens which proceeds anteriorly in midline and becomes an elongated, convoluted seminal vesicle. Cirrus pouch 0.325 to 0.400 by 0.105 to 0.124. Two accessory ducts and small accessory vesicles present in cirrus pouch, accessory ducts opening into ejaculatory duct of inverted cirrus (fig. 5); cirrus short, when everted presenting three papillae, larger central papilla bearing opening of ejaculatory duct, each lateral papilla bearing opening of an accessory duct (fig. 4).

Ovary subspherical, 0.130 to 0.294 by 0.155 to 0.216, lying mostly in right side of body; oviduct arising ventrally from ovary, joined by Laurer's canal, then bending toward right margin of body and sharply curving medially and ventrally to form conspicuous ootype; Laurer's canal sinuous, opening dorsally in midline. Vitellaria composed of large follicles (16 on right, 17 on left in one specimen), ventral to and partly overlapping ceca; right vitelline duct rather short, left vitelline duct longer; ducts uniting dorsal to Mehlis' gland and ventral to ovary to form prominent vitelline reservoir, which joins oviduct by means of short common vitelline duct immediately before ootype; Mehlis' gland compact, ventral and mostly posterior to ovary; uterus leaving Mehlis' gland area ventrally, terminating in relatively thin-walled metraterm. Eggs 0.025 by 0.012 (average of ten uterine

eggs, exclusive of polar filaments).

Host: *Pseudemys floridana* (Le Conte), the Florida terrapin.

Locality: Natural Bridge Sink, St. Mark's River, Leon County, Florida.

Types: Holotype, U.S.N.M. Helminth. Coll. No. 39057; paratypes, U.S.N.M. Helminth. Coll. No. 39058, 39059.

Incidence of Infection: Two of seven turtles parasitized.

Observations on Other Species of the Genus

Types and/or paratypes of *M. obtusicaudum*, *M. eversum*, and *M. kepneri* were examined in the course of this study. It is necessary to make the folowing additions to the descriptions of these three species.

Macravestibulum obtusicaudum Mackin, 1930

Mackin did not describe accessory vesicles and ducts in the cirrus pouch of this species, but Horsfall (1935) reported the presence of "three small gland-like bodies" in the cirrus pouch connected by ducts to the ejaculatory duct. She further stated: "These structures were visible but inconspicuous in Mackin's specimens which the author studied in 1933." The present author has studied a sectioned paratype of *M. obtusicaudum* from Dr. Mackin's private collection which shows in clear detail two vesicles only, as well as the structure of the everted cirrus (fig. 6). The cirrus presents three papillae, the central one being much the longest and bearing the opening of the ejaculatory duct. The other two papillae are armed with wart-like knobs and each papilla bears the opening of a duct leading to an accessory vesicle in the cirrus pouch. The writer has also examined two of Horsfall's specimens. One sectioned specimen (U.S.N.M. Helminth. Coll. No. 42037), an immature worm from *Graptemys pseudogeographica* from Reelfoot Lake, Tennessee, showed *four* rudimentary vesicles in the wall of the ejaculatory duct. The other, a whole mount (U.S.N.M. Helminth. Coll. No. 42038) of a 5-day-old worm from an experimental infection in *Graptemys geographica*, does not show these vesicles. Differences between the cirrus pouches of Horsfall's and Mackin's specimens may indicate that these worms are not conspecific, as believed by Horsfall; although it may be possible that two of the four original accessory vesicles may degenerate during maturation of the worm, resulting in a definitive condition of two. This could explain her observation of three accessory vesicles.

Horsfall seems to be in error regarding the nature of the excretory pore. A connection between the excretory bladder and the vestibular cavity was not seen by Mackin. Horsfall (1935) claimed to be able to "demonstrate 3 to 6 tiny ducts leading posteriad from the most posterior median part of the bladder to the vestibule" in thin sections of her specimens; and added this observation to the description of *M. obtusicaudum*. In the holotype of *M. obtusicaudum* (U.S.N.M. Helminth. Coll. No. 51698), however, a single opening between the excretory bladder and the vestibular cavity is evident. Furthermore, Horsfall's specimen mentioned above (No. 42037) also shows the condition of having a single opening between the bladder and vestibule.

It seems possible that *Carcaria infracaudata* Horsfall, 1930, is not the cercaria of *M. obtusicaudum* as maintained by Horsfall (1935). Besides the differences between the cirrus pouches of Mackin's and Horsfall's specimens, it is of importance to note that Horsfall did not describe a complete life history from one locality. Cercariae were obtained from *Goniobasis livescens* from the Vermilion River, Illinois, and experimentally fed to turtles; a few worms developed to maturity. Immature and mature worms were recovered from several species of turtles (*Pseudemys* and *Graptemys* from Reelfoot Lake, Tennessee. Thus, the validity of the life cycle of *M. obtusicaudum* as reported by Horsfall is open to question.

Macravestibulum eversum Hsü, 1937

Hsü (1937) described the cirrus and cirrus pouch of *M. eversum*, but did not indicate accessory vesicles. Examination of her type (U.S.N.M. Helminth. Coll. No. 55343) revealed in this species also two accessory vesicles and ducts in the cirrus pouch. The accessory ducts open into the ejaculatory duct. The appearance of the everted cirrus is unknown except for Hsü's brief description of its being short and capable of protrusion for a short distance only. Neither Hsü's figure nor her description of the egg of *M. eversum* indicates the presence of an operculum. Uterine eggs of the type specimen show the operculate condition typical for the genus.

Macravestibulum kepneri Jones, Mounts and Wolcott, 1945

The description of *M. kepneri* by Jones et al

(1945) included the statement that the Laurer's canal is "a vestigial pocket dorsal to the oviduct." A well-developed Laurer's canal which opens on the dorsal surface of the worm can be clearly seen in several of a series of sectioned paratypes. These authors also reported that the metraterm is serially ringed with five sphincters. A study of several sectioned paratypes revealed that the metraterm is rather long and not very heavily muscularized, similar to the condition found in *M. obtusicaudum* and *M. kraatzi*. The appearance of "sphincters" was apparently due to local contractions in the wall of the metraterm.

Comparison of M. kraatzi *with Other Species* in the Genus

Macravestibulum kraatzi differs from *M. obtusicaudum* in several ways, most conspicuously in the structure of the cirrus. The cirrus papillae of *M. kraatzi* are smooth as compared with the knobbed papillae of *M. obtusicaudum* (cf. figs. 4 and 6). *M. kraatzi* further differs from *M. obtusicaudum* in having a relatively smaller cirrus pouch and a vestibular cavity lacking prominent diverticula from the lateral pouches.

Macravestibulum kraatzi is distinguished from *M. eversum* mainly by body shape and size, *M. kraatzi* being longer and proportionately narrower; and by the fact that its metraterm is relatively thin-walled as compared to the conspicuous metraterm of *M. eversum*.

M. kraatzi differs from *M. kepneri* mainly in the structure of the cirrus. With the cirrus inverted, the accessory ducts in *M. kraatzi* open into the ejaculatory duct. In *M. kepneri*, the accessory ducts open on the distal surface of the inverted cirrus (Jones et al, 1945; plate 1, fig. 2). Also the accessory ducts in *M. kepneri* are greatly convoluted and the accessory vesicles are relatively much larger than those of *M. kraatzi*. *M. kepneri* also appears to be a more slender worm than is *M. kraatzi*, and to have considerably fewer uterine coils.

Revision of the Genus Macravestibulum Mackin, 1930

Since neither the original generic diagnosis by Mackin nor subsequent diagnoses by Ruiz (1946) and Yamaguti (1958) describe the cirrus complex and the bladder-vestibule connection adequately, the following revision is presented:

Macravestibulum Mackin, 1930, revised

Diagnosis: Pronocephalidae Looss, 1902; Choanophorinae Caballero, 1942, as revised by Ruiz (1946). Small trematodes, concave anteroventrally, with lateral margins of body roughly parallel. Anterior extremity lanceolate; cephalic collar present, incomplete ventrally. Posterior extremity truncate or rounded. Body wall strongly muscular anteriorly, weaker posteriorly. Cuticle smooth. Oral sucker small, subterminal; pharynx absent; esophagus long, slender. Ceca simple, terminating at or slightly behind level of posterior testis, with posterior extremities inflated and curving toward midline. Testes rounded, ventral to ceca, lying obliquely to each other, left testis anterior and dorsal to right. Cirrus pouch large, oblique; enclosing pars prostatica, small distal part of seminal vesicle, and two (possibly more) accessory vesicles and ducts; accessory ducts opening into ejaculatory duct or on distal surface of inverted cirrus, ducts opening separately from ejaculatory duct in everted cirrus. Cirrus short, opening into common genital atrium located in anterior third of body, intercecal, to left of midline. Vesicula seminalis externa long, convoluted. Ovary rounded, dorsal and anterior to testes, varying from slightly to right of midline to mostly in right side of body. Mehlis' gland ventral to ovary, compact, varying from directly beneath ovary to completely behind it. Laurer's canal present. Uterus mainly within intercecal field, sometimes overlapping ceca, forming numerous transverse loops, terminating with metraterm. Vitellaria composed of few large follicles, anterior to testes, overlapping ceca ventrally. Eggs with stout bipolar processes, base of one forming an operculum. Main part of excretory bladder "U"-shaped, with attenuated arms uniting anteriorly ventral to esophagus and usually continuing forward as a short median canal. Large posterior vestibular cavity present, bifurcated, eversible, lined with a thin cuticle, and communicating with excretory bladder by means of a median pore. Vestibular cavity opening to exterior by a large transverse slit. Intestinal parasites of fresh-water turtles (Emydidae).

Type species: Macravestibulum obtusicaudum Mackin, 1930

Subfamily Affinity of *Macravestibulum* Mackin, 1930

Price (1931), in his study of the family Pronocephalidae, recognized three subfamilies; Pronocephalinae Looss, 1899; Opisthoporinae Price, 1931; and Charaxicephalinae Price, 1931. *Macravestibulum* Mackin, 1930 was not included in his system.

In his review of the family, Mehra (1932) placed *Macravestibulum* in Pronocephalinae.

Caballero (1942) erected the subfamily Choanophorinae for *Choanophorus rovirosai*. This species possesses a bifurcated eversible vestibular cavity much like that of *Macravestibulum*, but the possession of a cuticular copulatory pouch sepa-

rates this form from members of the genus *Macravestibulum*. The excretory system proper is unknown.

In their description of *M. kepneri*, Jones, et al (1945) assigned the genus to the subfamily Choanophorinae Caballero, 1942; basing their decision on the possession of "the same broad distinctive characters" of the genera *Marcravestibulum* and *Choanophorus*.

Ruiz (1946) revised Choanophorinae Caballero, 1942, to include four genera: *Choanophorus* Caballero, 1942; *Macravestibulum* Mackin, 1930; *Metacetabulum* Teixiera de Freitas and Lent, 1938; and *Cetiosaccus* Gilbert, 1938. These genera show in common many characteristics, the most striking being the vestibular cavity or secondary excretory bladder. Ruiz presented a key to these genera; a translation from the Portuguese follows:

1. Cephalic collar absent; eggs without polar filaments.
   Metacetabulum Teixeira de Freitas and Lent, 1938
   Cephalic collar present; eggs with polar filaments 2
2. Vestibular cavity simple, testes tandem.
   Cetiosaccus Gilbert, 1938
   Vestibular cavity bifurcated, testes juxtaposed 3
3. Cuticular copulatory pouch present.
   Choanophorus Caballero, 1942
   Cuticular copulatory pouch absent.
   Macravestibulum Mackin, 1930

Yamaguti (1958) erected separate subfamilies for each genus listed above except *Choanophorus* which was already in Choanophorinae. He indicated in the generic diagnosis for *Metacetabulum* that the excretory system is unknown. His only reference to the vestibular cavity was the words "posterior portion retractile," Ruiz (1946) however, made a careful study of the excretory system of *Metacetabulum invaginatum* Teixeira de Freitas and Lent, 1938, and showed that the excretory system is quite similar to that found in *Macravestibulum* except for the fact that the vestibular cavity is not bifurcated. *Cetiosaccus galapagensis* Gilbert, 1938, was originally described as having a large, voluminous excretory vesicle with thick, folded walls which occupies the posterior fifth of the body or more, and is divided behind the cecal ends into long simple limbs anteriorly united. Ruiz must have rightly considered the thick walled bladder to be a vestibular cavity since he included *Cetiosaccus* in Choanophorinae. Gilbert's figure of *Cetiosaccus* clearly indicates a vestibular cavity much like that found in *Metacetabulum*.

The writer is of the opinion that the possession of a secondary excretory bladder, a character unique to the four genera *Choanophorus, Metacetabulum, Cetiosaccus,* and *Macravestibulum,* indicates a close phylogenetic relationship. Furthermore, the differences appearing in these genera are not sufficient to warrant their separation into four subfamilies. Therefore, these genera should be included within a single subfamily, Choanophorinae Caballero, 1942, as revised by Ruiz, 1946. The subfamilies Cetiosaccinae Yamaguti, 1958, Macravestibulinae Yamaguti, 1958, and Metacetabulinae Yamaguti, 1958, should be suppressed.

ACKNOWLEDGMENTS

The author is grateful to Dr. Robert B. Short under whose direction this work was done, to Mr. Dwayne N. Kruse for collecting and fixing two of the worms, and to Drs. Arthur W. Jones, George R. La Rue, Allen McIntosh and John G. Mackin for loan of type material.

This study was done under tenure of a National Science Foundation Cooperative Graduate Fellowship and a National Institutes of Health Predoctoral Fellowship.

SUMMARY

1. *Macravestibulum kraatzi* n. sp. is described. This species differs from others in the genus mainly in the character of the cirrus pouch, cirrus, metraterm, and body shape.
2. Additions to the descriptions of *M. obtusicaudum* Mackin, 1930, *M. eversum* Hsü, 1937, and *M. kepneri* Jones, Mounts and Wolcott, 1945, are made.
3. The diagnosis of the genus *Macravestibulum* Mackin, 1930 is revised to include new information on the cirrus pouch and cirrus.
4. The genus is returned to the subfamily Choanophorinae Caballero, 1942, as revised by Ruiz, 1946; and the subfamilies Cetiosaccinae Yamaguti, 1958, Macravestibulinae Yamaguti, 1958, and Metacetabulinae Yamaguti, 1958, are suppressed.

LITERATURE CITED

CABELLERO Y C., E. 1942 Tremátodos de las tortugas de Mexico. II. Descripción de un nuevo genero de la familia Pronocephalidae Looss, 1902 y descripción de una nueva especie del genero *Octangioides* Price, 1937. An. Inst. Biol. Univ. Nac. México **13**: 81–90.

DAMIAN, R. T. 1960 A new species of *Macraves-*

tibulum (Trematoda: Pronocephalidae) from the Florida Terrapin. A.S.B. Bull. **7**: 26.

HORSFALL, M. W. 1930 Studies on the structure of *Cercaria infracaudata* n. sp. J. Parasit. **17**: 43–48.

———. 1935 Observations on the life history of *Macravestibulum obtusicaudum* Mackin, 1930. (Trematoda: Pronocephalidae). Proc. Helm. Soc. Wash. **2**: 78–79.

HSÜ, D. Y. 1937 Life history and morphology of *Macravestibulum eversum* sp. nov. (Pronocephalidae, Trematoda). Tr. Am. Micr. Soc. **56**: 478–504.

JONES, A. W., MOUNTS, B. W. AND WOLCOTT, G. B. 1945 *Macravestibulum kepneri* n. sp.; a morphological and cytological study of a pronocephalid trematode. J. Morph. **77**: 285–297.

MACKIN, J. G. 1930 A new pronocephalid monostome from a freshwater turtle. J. Parasit. **17**: 25–29.

MEHRA, H. R. 1932 Classification de la famille des Pronocephalidae Looss. Ann. Parasit. **10**: 323–329.

PRICE, E. W. 1931 Redescription of two species of trematode worms from the MacCallum collection, with a note on the family Pronocephalide. Proc. U. S. Natl. Mus. (2865), v. **78**: Art. 22, pp. 1–10.

RUIZ, J. M. 1946 Pronocephalidae (Trematoda). Estudos das espécies brasileiras e revisão da família. Mem. Inst. Butantan **19**: 249–372.

YAMAGUTI, S. 1958 Systema Helminthum. Volume I. The digenetic trematodes of vertebrates, Parts I and II. Interscience Publishers, Inc., New York.

EXPLANATION OF PLATE I

All figures were drawn from fixed and stained specimens with the aid of camera lucida or microprojector. Scales are in millimeters.

Abbreviations: av, accessory vesicle; c, intestinal cecum; cd, common vitelline duct; cp, cirrus papilla; eb, excretory bladder; ej, ejaculatory duct; ga, genital atrium; le, Laurer's canal; le, lateral excretory canal; mg, Mehlis' gland; mt, metraterm; od, oviduct; ot, ootype; ov, ovary; sv, seminal vesicle; t, testis; v, vestibular cavity; vd, vitelline duct; ve, vas efferens; vf. vitelline follicles; vr, vitelline reservoir; u, uterus.

Macravestibulum kraatzi n. sp.

FIGURE 1. Ventral view of holotype drawn from whole mount.

FIGURE 2. Dorsal view of female genital complex, reconstructed from frontal sections.

FIGURE 3. Uterine egg.

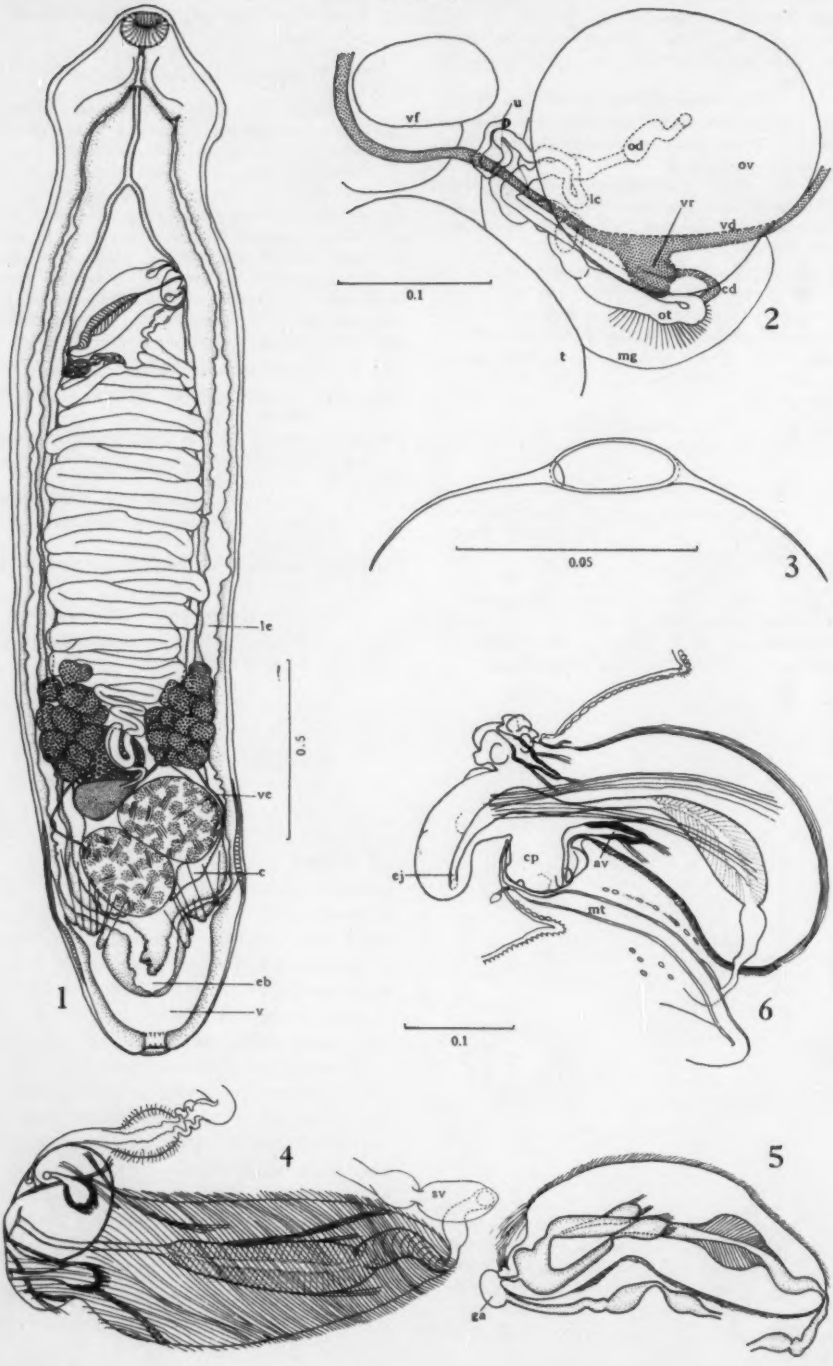
FIGURE 4. Partially everted cirrus, showing metraterm, reconstructed from frontal sections.

FIGURE 5. Inverted cirrus, reconstructed from frontal sections.

Macravestibulum obtusicaudum

FIGURE 6. Everted cirrus, reconstructed from sagittal sections.

PLATE I


vf
u
p
od
ov
lc
vr
vd
0.1
cd
ot
mg
t
2
0.05
3
le
f
0.5
ve
c
eb
v
1
ej
cp
av
mt
0.1
6
4
sv
5
ga

PROTOPLASMIC PROCESSES OF EPITHELIAL CELLS OF *GORGODERA*

G. S. Tulloch, J. E. Shapiro and B. Hershenov
Department of Biology, Brooklyn College, Brooklyn 10, N. Y.

The existence of pseudopodial extensions on the intestinal epithelium of trematodes, specifically *Fasciola hepatica* L., has been known for many years, but only recently have they been the subject of intensive investigation by light and electron microscopy (Gresson and Threadgold, 1959). This note records the occurrence of similar epithelial modifications in both the sperm and uterine ducts of another trematode, *Gorgodera amplicava* Looss.

MATERIALS AND METHODS

Living specimens of *Gorgodera amplicava* were removed from the urinary bladders of *Rana pipiens* which had been killed by decapitation, washed in saline, fixed in veronal-buffered osmic acid following the method of Palade and embedded in n-butyl methacrylate. Sections were prepared using glass knives and a Porter-Blum mechanical advance ultramicrotome, and examined with an RCA (EMU-2B) electron microscope. Micrographs were taken at an initial magnification of 9,500 and enlarged photographically to the desired size. The magnification of the settings of the microscope was determined by calibration using a diffraction grating ruled at 28,800 lines to the inch.

RESULTS

Sperm ducts (figs. 1 and 2). Aside from sperm cells (S), the only other organized structures observable in the lumen are loop extensions (P) of the free ends of the epithelial cells (E). These form loose coils which are disposed along the wall (fig. 1) rather than extending freely toward the center of the sperm duct. Each loop is circular in cross section and has a constant diameter of 0.035 microns. The outer limiting double membrane (M) of each loop, the continuation of the free cell surface, is 60 A in thickness. The substance within the loop has the same organization as the cytoplasm of the parent cell.

The identity of these protoplasmic processes as loops rather than finger-like extensions is based on the occasional section in which the origin, course and termination of a single loop may be observed (fig. 2, A and B). Moreover, those structures which appear as finger-like extensions actually are portions of protoplasmic loops (TP).

Uterus (figs. 3 and 4). This organ, readily recognizable by eggs within the lumen, has an epithelium which in certain portions bears protoplasmic processes. At the level in which very young eggs are present (fig. 3), the pseudopodial extensions are long and extend well into the lumen. In regions where more mature eggs are found, the processes are reduced in length leaving a clear lumen (fig. 4). As in the case of the sperm duct, the protoplasmic extensions of the uterus arise and terminate in the epithelial surface and, therefore, form loops. The loops may coil extensively in some areas (fig. 3) and be relatively uncomplicated in others (fig. 4). The cross-sectional dimension of the uterine processes is the same as those of the sperm duct (0.035 microns).

Occasionally there are protoplasmic processes (OP) observed on the young eggs (O) which are morphologically identical with the uterine extensions of the contiguous area. Whether these processes are portions of protoplasmic loops of an egg or a part of a structure connecting an egg with the uterine wall has not been determined.

DISCUSSION

Modifications of the free ends of epithelial cells are many and varied. In their work on the gut epithelium of *Fasciola*, Gresson and Threadgold noted the tremendous increase in free cell surface provided by thousands of protoplasmic processes, some of which were in the form of loops, and ascribed to them an absorptive function. Yasazumi et al (1960) observed epithelial extensions in the seminiferous tubules of the pond snail, *Cipangopaludina malleata* Reeve, which are not unlike those of *Fasciola*, to which they attributed a secretory role, since the cells of origin were presumed by them to be functionally related to the Sertoli or "nurse cells"

Received for publication October 20, 1960.

found in other forms.

The observations reported here show that epithelial modifications in the form of protoplasmic loops which provide a great increase in the amount of free cell surface are prominent in both the sperm duct and uterus of *Gorgodera*. The occasional appearance of an extension from an egg suggests the existence of at least a transient surface-increasing device. There is no overwhelming evidence which would favor a principal role of either absorption or secretion, although the graduated series of uterine extensions would favor a secretory or nutritive role during the period before the egg shell had completely hardened. It is well established that the surfaces of cells are capable of regulating molecular transport in either direction. Since the protoplasmic loops are merely devices to carry expanded cell membranes, a conservative statement of their role might be simply to permit greater molecular transfer without reference to direction.

References

GRESSON, R. A. R. AND THREADGOLD, L. T. 1959 A light and electron microscope study of the epithelial cells of the gut of *Fasciola hepatica* L. J. Biophysic. and Biochem. Cytol. **6**: 157–162.

YASAZUMI, G., HIROAKI, T. AND TEZUKA, O. 1960 Spermatogenesis in animals as revealed by electron microscopy. VIII. Relation between the nutritive cells and the developing spermatids in a pond snail, *Cipangopaludina Malleata* Reeve. J. Biophysic and Biochem. Cytol. **7**: 499–504.

Explanation of Plate I

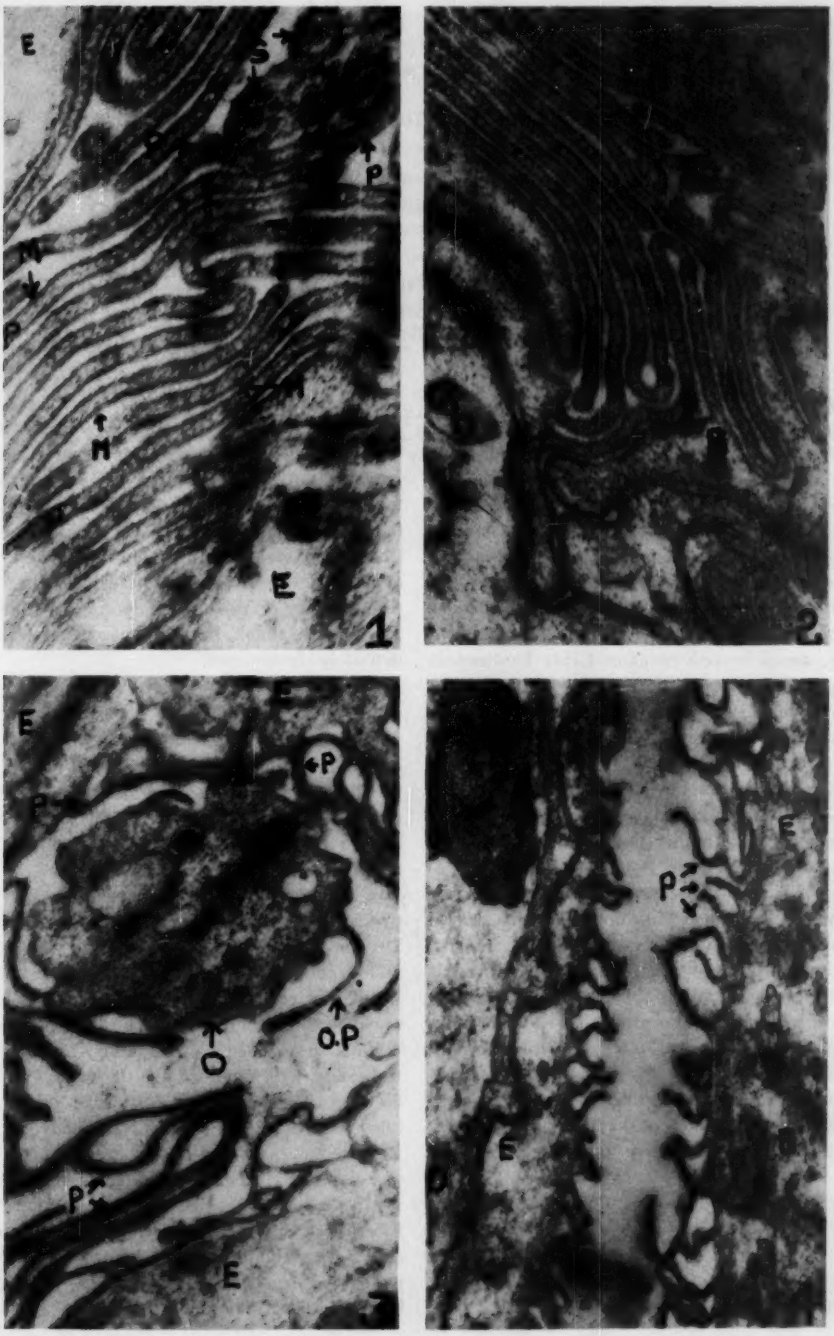
FIGURE 1. Section through sperm duct showing the epithelial surface (E) and its double membraned (M) extensions (P) surrounding sperm cells (S). Protoplasmic loops which are observed in section appear as finger-like processes (TP). × 70,000.

FIGURE 2. Section of sperm duct showing origin (A) and termination (B) of a single protoplasmic loop. × 50,000.

FIGURE 3. Section of uterus in the proximal region showing the protoplasmic extensions (P) of the epithelium (E) as well as a protoplasmic process (OP) extending from the egg (O). × 33,000.

FIGURE 4. Section of the distal portion of the uterus showing protoplasmic loops (P) arising from the epithelium (E). × 33,000.

PLATE I



NOTES ON *SANGUINICOLA DAVISI* (TREMATODA: SANGUINICOLIDAE) IN THE GILLS OF TROUT

H. S. Davis*

Prepared from Dr. Davis' notes by

G. L. Hoffman

Bureau of Sport Fisheries and Wildlife, Eastern Fish Disease Laboratory, Leetown (P.O. Kearneysville), W. Va.

and

E. W. Surber

Bureau of Sport Fisheries and Wildlife, Branch of Federal Aid, Peachtree-Seventh Building, Atlanta 23, Ga.

In 1953 the late Dr. Davis recorded the presence of an unnamed trematode from the gill blood vessels of fingerling cutthroat trout (*Salmo clarki*) and fingerling steelhead trout (*Salmo gairdneri*) at the Alsea Hatchery and in yearling rainbow trout (*Salmo gairdneri*) at the Oakridge Hatchery, both of the Oregon State Game Commission. Subsequently, two blood flukes, *Sanguinicola davisi* and *S. klamathensis*, were described from trout by Wales (1958). The present species was determined to be *S. davisi* by one of us (GLH). This report supplements data reported by Davis (1953) and Wales (1958). Unfortunately, Dr. Davis' illustrations for the present paper could not be found.

While Dr. Davis was examining the gills of fingerling cutthroat trout, he noticed numerous peculiar bodies in the capillaries which resembled superficially the vegetative state of a myxosporidian. However, the true nature of the organism remained in doubt until several months later when free-swimming miracidia emerged from the gills. Later, several adult worms were found in the arteries of the gill arches where they were difficult to find because of their small size and number. Although only certain stages of the life-cycle were available for study, it is believed that the information at hand regarding this remarkable worm is of sufficient interest to justify publication at this time.

In addition to cutthroat trout, the parasite also occurred in the gills of fingerling steelhead trout, *Salmo gairdneri*, at the same hatchery, but was not so abundant as in the cutthroat trout. An adult worm and cysts were found in the gills of a yearling rainbow trout from the Oakridge Hatchery. It is believed, on the basis of reports that reached Dr. Davis, that this parasite may occur in other species of trout and also young salmon, but this has not as yet been definitely determined.

Description of the Adult Worm

The adult worm was available for study only in sections. It has been found only in the arteries which extend from the heart to the gill arches and, owing to its minute size, it was very difficult to find the worms by dissection of fresh material. This difficulty was increased by the fact that the adult worms were apparently not present in large numbers. It is described in Dr. Davis' notes as being strongly contractile and leaflike, resembling a typical planarian worm. It is very small, the largest observed being only about 650 microns in length. Another individual was 435 microns long, 110 microns wide, and with a maximum thickness of about 55 microns. Numerous spines are present in the body wall along each side of the body.

The mouth at the anterior end opens into the esophagus which is dilated and resembles a pharynx. It is separated by a constriction from the rest of the alimenary canal which extends as a slender tube for some distance and then expands to form a sac-like structure constricted in the middle. The wall of the canal is moder-

Received for publication November 22, 1960.

* The late H. S. Davis was formerly Fish Pathologist In-charge, Aquiculture Investigations, U. S. Fish and Wildlife Service, and author of "Culture and Diseases of Game Fishes."

ately thick, but there is no indication of a cellular structure other than the presence of an occasional nucleus.

The irregular-shaped testes lie near the middle of the body and can be easily distinguished by the large numbers of thread-like spermatozoa interspersed with germ cells in various stages of development. The vas deferens follows a sinuous course from the testes to the external opening at the posterior end. The posterior two-thirds of the semnial vesicle is lined with large conspicuous cells containing coarse granules that stain deeply with eosin. It was impossible to determine if a cirrus was present. Both genital openings are close together in a shallow groove near the posterior end of the body.

The ovary is convoluted just posterior to the testes. The oviduct is at first small, thin-walled, and difficult to distinguish, but after being joined by the vitelline duct it expands into a sac-like organ constricted in the middle. This sac extends posterior then doubles on itself to reach the genital pore. The vitellaria are distributed throughout most of the body and fill much of the space not occupied by the testes and ovary.

The excretory vesicle was not determined. The protonephridia described by Wales (1958) for this worm may be the excretory vesicle.

The Ovum and Micracidium

The entire development of the micracidia takes place in the gills where they form cysts within the capillaries. Since the adult worms live in the ventral aorta and branchial arteries, the eggs when deposited in the circulating blood are carried directly to the gills where most of them become lodged in the capillaries. A few may continue their development attached to the walls of the large blood vessels. At this stage they might easily be confused with the trophozoite stage of myxosporidea. Two relatively large vitelline cells became associated with each primary oocyte so closely that cell outlines were indistinguishable. The nucleus of the oocyte can, however, be easily distinguished by its considerably larger size and large central plasmosome. Each vitelline cell nucleus contains a smaller and usually eccentric plasmosome. Both types of nuclei have a well defined chromatic network. There is no shell around the egg as in other species of *Sanguinicola*. The zygote passes through the early cleavage stages rapidly but the vitelline cells do not divide for some time and can be easily recognized as two large cells which are attached to opposite sides of the embryo. In it the cytoplasm surrounding the nuclei is clear and stains more deeply than in the vitelline cells.

The further history of the two types of cells is difficult to trace. Both types multiply by mitoses but the vitelline cells are always sharply set off from the embryo and take no part in its development except to act as nurse cells. Their principal function is to form the wall of the "cyst" in which the miracidium develops. Differences between the vitelline cell nuclei and those of the embryo become even more striking. The latter vary greatly in size and structure while the vitelline cells become flattened to form the "cyst," a continuous layer one cell thick around the growing embryo.

The miracidium in its "cyst" is ovoid and may be conical at one end. The length of the miracidia preserved in formalin was 33 to 37 microns and the width 24 to 27 microns. Its surface bears long cilia, apparently uniformly distributed and is marked with fine longitudinal striations. The most striking change, however, is in the absence of most of the nuclei which were present in younger embryos but which apparently disappear as development proceeds; only 20 nuclei remain, 18 in a rounded mass of denser protoplasm at one end of the miracidium and just anterior to them two small nuclei. The most conspicuous feature of the living miracidium is the pigmented "eye spot" which is median and just anterior to the nucleated mass.

At the conical anterior end of the body is a stylet which extends into the body at an angle and ends near the "eye spot." The stylet stains only faintly with Ehrlich's hematoxylin but intensely with iron hematoxylin. In cross section it appears to be a flattened tube, tapered somewhat toward the rounded anterior end. Its wall has several longitudinal thickenings which stain more deeply than the remainder and have the appearance of the longitudinal bands described in *Sanguinicola inermis* by Plehn (1924). At the anterior end of the stylet, the protoplasm appears to be clearer and stains more deeply which may indicate the presence of contractile elements. The stylet probably is extended a short distance from the body when in use. Plehn thought that the stylet of *S. inermis* was used for piercing the egg shell, but since the "cyst"

in *S. davisi* is soft and easily separated, it appears probable that the stylet functions primarily in penetrating the tissues of the snail host.

The "cyst" wall is separated from the miracidium by a narrow space and varies in thickness, but as the miracidium matures it becomes considerably thinner. It contains three types of nuclei, the most conspicuous being a number of large flattened nuclei, oval in section and containing a large plasmosome in addition to the chromatin network. There are also several rounded nuclei filled with coarse chromatin granules, but without a plasmosome. Two of these are usually larger than the others, and are located close together and are surrounded by granular cytoplasm. The third type of nucleus is small, greatly elongated and in various stages of degeneration. It is probable that these are nuclei of endothelial cells which have become closely attached to the "cyst" wall. The "cysts" vary somewhat in size with an average length of 40 microns and a width of 27 to 30 microns in fixed material. The miracidium emerges by rupturing the "cyst" wall at one end.

Effect on the Host

There is no evidence that the adult worms materially injure the host. They are so minute and occur in such small numbers that they have little if any effect on the blood vessels or blood. The developing miracidia, however, may cause serious injury to the gills and often heavy mortality among infected fingerlings. A few embryonic stages in the gills are of little consequence but, where abundant, as they frequently are, it is a very different story. Since the adult worms evidently deposit large numbers of eggs in the blood stream, the presence of even two or three worms may result in the gills becoming fairly riddled with embryos in various stages of development. It is by no means exceptional to find several embryos in a single lamella, resulting in breakdown of gill tissues. Heavily infected fish are also noticeably anemic (Method of determining anemia was not stated).

Evidently, an occasional egg is carried past the gills since a few embryos are found in the kidneys, spleen, and even in the walls of the heart but may never be so abundant in such organs as to cause appreciable injury. Moreover, such embryos are in a blind alley as far as their future development is concerned and most of those observed were in various stages of disintegration.

SUMMARY

The blood fluke, *Sanguinicola davisi*, was found in the blood vessels of the gill arches of *Salmo clarki* and *Salmo gairdneri* in Oregon. The development of the miracidium and its associated "cyst" is described. Heavy mortalities resulted from the damage done to the gills by the presence of the developing miracidia.

ACKNOWLEDGMENT

Dr. F. G. Wallace, Zoology Department, University of Minnesota, Drs. S. F. Snieszko and Ken Wolf, Eastern Fish Disease Laboratory, Leetown (P.O. Kearnesyville), West Virginia have kindly read the manuscript.

LITERATURE CITED

DAVIS, H. S. 1953 Culture and Disease of Game Fishes. Univ. Calif. Press, Berkeley. 352 pp.

PLEHN M. 1924 Praktikum der Fischkrankheiten. E. Schweizerbart, Stuttgart. 179 pp.

WALES, J. H. 1958 Two new blood fluke parasites of trout. Calif. Fish and Game 44: 125–136.

NEW ASPIDOGASTRID TREMATODE, *COTYLASPIS REELFOOTENSIS*, FROM SOME TENNESSEE MUSSELS*

HAIG H. NAJARIAN

Department of Microbiology, The University of Texas Medical Branch, Galveston, Texas

It is generally accepted that the subclass Aspidogastrea Faust and Tang, 1936 represents a group of worms intermediate in morphology and life history between monogenetic and digenetic trematodes. This paper describes a new aspidogastrid species, *Cotylaspis reelfootensis*, n. sp., from some mussels (listed below) collected in the Bayou du Chien region of Reelfoot Lake, Tennessee. Its anatomical features are compared with other species previously described in the genus *Cotylaspis* Leidy 1857: *C. insignis* Leidy 1857, *C. lenoiri* Poirier 1886, *C. cokeri* Barker and Parsons 1914, *C. stunkardi* Rumbold 1927, and *C. sinensis* Faust and Tang 1936.

MATERIALS AND METHODS

The pelecypods were collected by hand and the soft parts exposed by cutting the adductor muscle with a scalpel. The mantle, mantle cavity, gills, foot, visceral mass, and kidney-heart region were then inspected under a dissecting microscope for the presence and location of worms. Worms were removed either by a wide-mouth medicine dropper or by an L-shaped dissecting needle, and then placed in tap water or saline. Morphological observations were made mostly on mounted living specimens with a minimum of cover glass pressure. Worms were also fixed in hot A.F.A. or 10 percent formalin and later stained with Mayer's HCL carmine or Delafield's hematoxylin. Material for sectioning was fixed and stained in a similar manner. All average and range measurements given are in millimeters and are based on 25 specimens fixed in hot 10 percent formalin and mounted in toto.

Cotylaspis reelfootensis, n. sp.

Diagnosis: Aspidogastrid trematode with characters of genus; body elongate, 0.7 to 2.2 long (average 1.7); ventral sucker large, 0.5 to 1.2 long (average 1.1) and 0.5 to 0.9 (average 0.8) wide; ventral sucker divided into 29 alveoli (20 peripheral and 9 medial); 40 marginal organs, 20 on dorsal and 20 in medial borders of peripheral rim of alveoli; cuticula aspinose; body wall with three layers of muscle, circular, longitudinal and oblique; oral sucker lacking; mouth at tip of eversible oral cone (diameter 0.3) capable of assuming many shapes; pharynx large, protrusible, diameter 0.15; paired eye spots 0.016 by 0.007 at anterior end of pharynx; prepharynx and esophagus lacking; intestine single, sacculate (average length 1.4, average width 0.15) extending almost to posterior end of body, intestinal epithelium very glandular; excretory pore single, dorsal, posterior; excretory bladder slightly bilobed (0.17 by 0.08) just posterior to intestine; excretory system stenostomate, main excretory collecting ducts branching into anterior and posterior secondary collecting ducts near mid-length of body; anterior secondary collecting vessel arising near oral cone in three capillaries, each ending in a single flame cell; posterior secondary collecting duct arising in several collecting tubules with complex branching; several capillaries and flame cells observed, always in groups of three; ciliated tufts in both arms of main collecting ducts mostly in second fourth of body; testis single, large, spherical, 0.25 in diameter, anterior and to right of end of intestine; vas efferens single, narrow, at right of intestine, extending anteriorly and widening into sinuous seminal vesicle (usually filled with spermatozoa), latter crossing over to left side at one-third level of body in entering cirrus sac; cirrus sac muscular, 0.165 long and 0.10 wide, at left of intestine; cirrus and prostate gland cells absent; cirrus sac entering genital atrium, latter located slightly to left of mid-line, some distance posterior to pharynx; genital atrium muscular, opening ventrally by single genital pore; vitelline follicles large, 0.45 to 0.6 in diameter, in posterior two-thirds of body, located mostly laterally; ovary small (0.066 in diameter) on left side about mid-level of body; oviduct short; ootype and Mehlis' gland present; seminal receptacle absent; large vitelline reservoir on left side just posterior to ootype; common vitelline duct entering oviduct just before and to left of ootype; uterus thin-walled, very elastic, capable of narrowing to extremely small diameter; ascending loop of uterus on left side; uterus then bending posteriad and running ventral to posterior end of intestine, crossing over to right side of body and extending anteriorly on right side of body, entering genital atrium from right just posterior to pharynx; eggs large, 0.165 to 0.180 long (average 0.168) by 0.92 to 0.115 wide (average 0.98), kidney-shaped and operculate at narrower end; average of 6 eggs per

Received for publication November 14, 1960.

* Part of this study was supported by the Reelfoot Lake Biological Station of the Tennessee Academy of Science. Appreciation is extended to Dr. Allen McIntosh, Beltsville Parasitological Laboratory, U. S. D. A., for lending specimens of *C. insignis* and *C. cokeri*.

mature worm; eggs in uterus undeveloped or at most in two-cell stage.

Hosts: *Anadonta grandis* Say, *Ligumia subrostrata* Say, and *Unionerus tetralasmus* Say.

Location in Hosts: Cavities bordering gills, heart, kidney, and foot.

Locality: Reelfoot Lake, Tennessee.

Type Specimens: Holotype and paratypes are deposited in the Helminthological Collection, U. S. National Museum; No. 39069.

DISCUSSION

The genus *Cotylaspis* was erected by Leidy (1857) with *C. insignis* as type species from mussels (Unionidae) collected in Pennsylvania. *C. reelfootensis* is the second species described from mussels, and the genus *Anodonta* is most frequently infected with this trematode (Najarian, 1955). There have been four other species described in the genus *Cotylaspis*, all from the small intestine of fresh-water turtles: *C. lenoiri* Poirier, 1886, from Africa, *C. sinensis* Faust and Tang, 1936, from China and *C. cokeri* Barker and Parsons, 1914, and *C. stunkardi* Rumbond, 1927, from the United States.

The most noticeable character which distinguishes *C. reelfootensis* from the other described species in the genus is the number and arrangement of the so-called marginal organs. There are 20 in *C. insignis*, none in *C. lenoiri*, 22 in *C. cokeri*, 34 in *C. stunkardi*, 18 in *C. sinensis*, and 40 in *C. reelfootensis*. When described, each marginal organ has been located at the distal border of each peripheral alveolus of the ventral sucker, whereas in *C. reelfootensis* in addition to 20 organs being located peripherally, 20 are also present on the medial side of each peripheral alveolus bordering on the medial alveoli. The exact nature of these organs has not been demonstrated. Dujardin (1845) in *Aspidogaster*, Looss (1902), and Osborn (1903) in *Cotylaspis* considered them as glandular, whereas Voeltzkow (1888) in *Aspidogaster*, Monticelli (1892) and Nickerson (1902) in *Cotylogaster*, and Stunkard (1917) in *Cotylaspis* regarded them as sensory structures. The marginal organs in *C. reelfootensis* are eversible, and contract in a manner similar to the excretory bladder. The bulbous portion of the organs never protrudes. Glandular concretions were observed both in the bulb and leaving the narrow channel to the exterior, indicating possible secretory function.

In general appearance *C. reelfootensis* most closely resembles *C. insignis* but, in addition to the number and arrangement of the marginal organs, differs from the latter in the absence of both prepharynx and esophagus, a slightly bilobed excretory bladder instead of in two parts, and the absence of a cirrus. Mehlis' gland and the ootype have not been described for *C. insignis* but are present in *C. reelfootensis*. In *C. insignis* ciliated tufts are present only in the main posterior collecting duct next to the pharynx, whereas in *C. reelfootensis* ciliated tufts are present in both ascending and descending limbs of the main collecting duct at the midlevel of the body. Osborn (1903) stated that the excretory capillaries ending in flame cells are never branched tricotyomously in *C. insignis*. In *C. reelfootensis* they always end in groups of three. In *C. insignis* the ovary is on the right side whereas in *C. reelfootensis* it is on the left. The testis of *C. insignis* is located more posteriorly than in *C. reelfootensis*. The concretionary bodies in the vas efferens of *C. insignis* where it expands into the seminal vesicle are absent from *C. reelfootensis*. There is disagreement as to whether or not *C. insignis* has a genital atrium. Stunkard (1917) stated the genital ducts open to the exterior separately, whereas Osborn (1903) observed a single genital atrium and pore into which the two ducts lead, the latter condition being present in *C. reelfootensis*. The single recurrent loop of the uterus of *C. reelfootensis* is distinctly different from the winding nature of the uterus of *C. insignis*.

C. cokeri differs from *C. reelfootensis* in the nature of the host, the number of alveoli on the ventral sucker, the number and arrangement of marginal organs, in the presence of an esophagus, cirrus, prostate gland, and double genital pore, the absence of a genital atrium, and the position of ovary and testis. Stunkard (1917) described an ootype in *C. cokeri* but did not designate it as such.

C. stunkardi differs from *C. reelfootensis* in the nature of the host, number of alveoli, number and arrangement of marginal organs, presence of esophagus, size, shape and position of ovary and testis, extent of vitellaria, convoluted nature of the uterus, and the greater average number of eggs per worm.

C. sinensis differs from *C. reelfootensis* in the nature of the host, number of alveoli, number and arrangement of marginal organs, size and position of ovary and testis, absence of ootype, presence of prostate gland and seminal

receptacle, and a small vitelline reservoir.

C. lenoiri differs from *C. reelfootensis* in the nature of the host, number of alveoli, absence of marginal organs, size and position of ovary and testis, the extent of the vitellaria, and the large size of the vitelline reservoir.

The systematics of aspidogastrid trematodes has been reviewed by Nickerson (1902), Stunkard (1917), Dawes (1941), and Dollfus (1956, 1958). The most definitive account was given by Dollfus (1958) who listed three characters which distinguish the genus *Cotylaspis* from other genera in the subfamily Aspidogastrinae: (1) three longitudinal rows of alveoli, (2) a single testis, and (3) a cirrus sac. Since the alveoli of the ventral sucker are not actually in three longitudinal rows, this character might better be re-stated as a peripheral rim of alveoli surrounding a single longitudinal row of alveoli.

SUMMARY

Cotylaspis reelfootensis, n. sp. is described from the mussels *Anadonta grandis* Say, *Ligumia subrostrata* Say, and *Uniomerus tetralasmus* Say collected from Reelfoot Lake, Tennessee. This is the sixth species described in the genus *Cotylaspis* Leidy and the second species of *Cotylaspis* reported from pelecypods. Its anatomical characters are compared with previously described species in the genus.

REFERENCES

BARKER, F. D. AND PARSONS, S. 1914 A new aspidobothrid trematode from Lesseur's terrapin. Tr. Am. Micr. Soc. **33**: 26–262.

DAWES, B. 1941 On *Multicotyle purvisi*, n.g., n. sp., an anspidogastrid trematode from the river turtle, *Sienbenrockiella crassicillis*, in Malaya. Parasitology **33**: 300–305.

DOLLFUS, R. P. 1956 Système de la sous-classe des Aspidogastrea Faust and Tang 1936. Ann. Parasit. **31**: 11–13.

———— 1958 Trematodes. Sous-Class Aspidogastrea. Ann. Parasit. **33**: 305–395.

DUJARDIN, F. 1845 Histoire Naturelle des Helminthes ou Vers Intestinaux. Paris.

FAUST, E. C. AND TANG, C. C. 1936 Notes on new aspidogastrid species, with a consideration of the phylogeny of the group. Parasitology **28**: 487–501.

LEIDY, J. 1857 Observations on Entozoa found in the Naiades. Proc. Acad. Nat. Sci. Phila. **10**: 18.

LOOSS, A. 1902 Ueber neue und bekannte Trematoden aus Seeschilkröten. Zool. Jahrb. Abt. Syst. **16**: 21–32.

MONTICELLI, F. S. 1892 *Cotylogaster michaelis*, ng., n. sp., e revisione delgi Aspidobothridae. Festschr. Leuckart, pp. 168–214.

NAJARIAN, H. H. 1955 Notes on aspidogastrid trematodes and hydracarina from some Tennessee mussels. J. Tenn. Acad. Sci. **30**: 11–14.

NICKERSON, W. S. 1902 *Cotylogaster occidentalis* n. sp. and a revision of the family Aspidobothridae. Zool. Jahrb. Abt. Syst. **15**: 597–624.

OSBORN, H. L. 1903 On the habits and structure of *Cotylaspis insignis* Leidy, from Lake Chautauqua, New York. J. Morph. **18**: 1–44.

POIRIER, J. 1886 Trématodes nouveaux ou peu connus. Bull. Soc. Phil. Paris **10**: 20–40.

RUMBOLD, D. W. 1927 A new trematode from the snapping turtle. J. Eli. Mitch. Sci. Soc. **43**: 195–198.

STUNKARD, H. W. 1917 Studies on North American Polystomidae, Aspidogastridae, and Paramphistomidae. Ill. Biol. Monog. **3**: 1–114.

VOELTZKOW, A. 1888 *Aspidogaster conchicola*. Arb. Zool. Zootom. Inst. Wurz. **8**: 249–292.

EXPLANATION OF PLATES

PLATE I

FIGURES 1–3. Sketches of *C. reelfootensis* from ventral, dorsal, and lateral aspects.

FIGURE 4. Single alveolus of ventral sucker with four marginal organs.

FIGURE 5. Optical section of marginal organ, showing protrusibility, and communication with exterior.

FIGURE 6. Three views of an eyespot.

FIGURE 7. *C. reelfootensis*, type specimen, dorsal view.

FIGURE 8. Optical section of pharynx and anterior portion of intestine.

FIGURE 9. Alveoli of posterior end of worm; dotted lines indicate positions of borders, before contraction.

FIGURE 10. Ventral sucker showing number and arrangement of alveoli and marginal organs.

PLATE II

FIGURES 11–14. Sketches of anterior end of worm showing various positions of pharynx in relation to oral cone.

FIGURES 15–17. Sketches of various shapes of oral cone.

FIGURE 18. Vitelline cells of single vitelline follicle.

FIGURE 19. Portion of uterus with single egg, indicating elasticity of uterus.

FIGURE 20. Optical section of uterus showing fibrous condition.

FIGURE 21. Incomplete sketch of excretory system showing main collecting ducts and their branches, ciliated tufts, and tripartite nature of capillaries and flame cells.

FIGURE 22. Optical section of body wall showing cuticle, paremchyma, and musculature.

FIGURE 23. Egg of *C. reelfootensis* after incubation at room temperature for 3 days; morula stage present and vitelline cells broken down.

FIGURE 24. Views of eggs from uterus showing germinal cells, vitelline cells, and opercula.

FIGURE 25. Sketches showing successive stages in operation of excretory bladder.

RESEARCH NOTE

ON THE ECOLOGY OF *LEIDYNEMA APPENDICULATA* (LEIDY, 1850) (NEMATODA: OXYUROIDEA).

During attempts to raise parasite-free *Blatta orientalis* for experimental purposes, nymphs hatching from isolated oothecae obtained from the Quartermaster Research and Engineering Center, Natick, Massachusetts, were found to be protozoa-free, but had very large numbers (up to 85 adults) of *Leidynema appendiculata*. This high infection occurred in subsequent experiments, even though the oothecae were rinsed in 50 percent alcohol before incubation. However, when the egg cases were cleaned with detergent (Tide) solution, the nymphs which hatched were *Leidynema*-free. The ability to obtain nematode-free *Blatta* only from thoroughly cleaned oothecae indicates that *Leidynema* eggs are transferred by adhering to the oothecae, as well as directly from adult to nymph. There is also some evidence that *Leidynema* eggs may occasionally be carried within roach oothecae.

It was further noted that average populations of *Leidynema* in protozoon-free *Blatta* were usually greater than those of protozoon infected hosts. One experiment was undertaken in which 12 protozoon-free *Blatta* were experimentally infected with *Nyctotherus oralis, Lophomonas striata,* and *L. blattarum*. Twelve roaches from the same source were isolated as controls. After 42 days of incubation the hosts of both groups were killed and the populations of *Leidynema* tabulated. Data are presented in table I.

TABLE I. *Relation of protozoon infection to populations of* Leidynema appendiculata *in* Blatta orientalis.

Host	*Blatta orientalis*					
	Stock culture (75 per jar) protozoon-free 30 examined		Control group (12 per jar) protozoon-free 11 examined		Experimentally protozoon-infected (12 per jar) 11 examined	
	Numbers of adult female *Leidynema* present					
Sex	Range	Ave.	Range	Ave.	Range	Ave.
Males	4–19	15	5–15	9.25	4–11	6
Females	2–85	24.7	6–31	15.3	2–19	11.2
Nymphs	1–18	8	5–18	8.5	1– 5	2.5
Totals	1–85	18.2	5–31	10.6	1–19	5.7

The smaller average number of *Leidynema* in both experimental and control groups of hosts compared with the protozoon-free stock culture is probably due to the decrease in contact between roaches under less crowded conditions. The further decrease of *Leidynema* in experimentally protozoon-infected hosts is apparently directly related to the presence of protozoa. Further investigations are required to determine the extent and basis of this limitation of *Leidynema* population by associated protozoa.

In a brief series of in vitro experiments on *Leidynema* it was found that addition of albumin and glucose to Ringer's solution extended the viability of the nematode by about 2 days. Microscopic examination of numerous living specimens failed to reveal whether the worms ingested either fluid or particulate material of their medium. In order to visualize particulate ingestion, more than 15 *Leidynema* were kept for periods of 24 hours in suspensions of Congo Red stained yeast or India ink. Since no ingestion of particulate matter was observed under these conditions, it is suggested that the worms gain nutrition by absorption.—C. M. FAY, *Department of Biological Sciences, University of Cincinnati.*

PLATE I

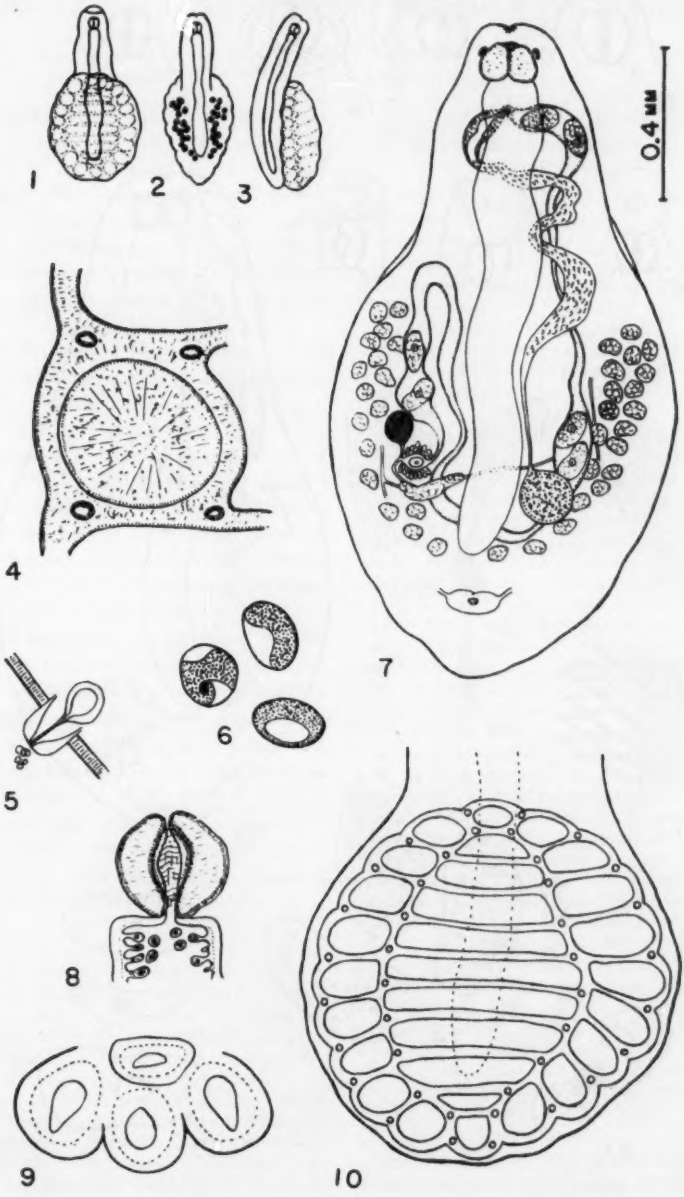
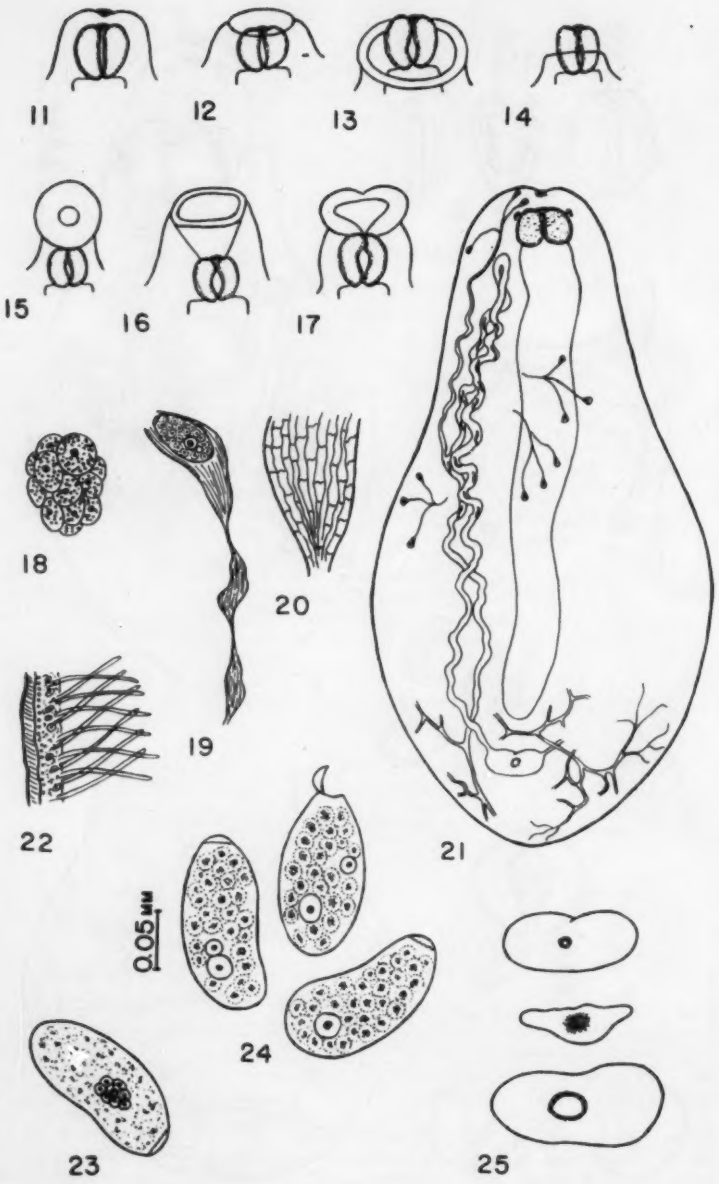


PLATE II



SOME DIGENETIC TREMATODES OF PHILIPPINE FOOD FISHES*

CARMEN C. VELASQUEZ
Department of Zoology, University of the Philippines

Our knowledge of trematodes of Philippine food fishes is quite limited. The check list (Tubangui, 1947) of parasitic worms reported from the Philippines shows no trematode collections from *Caranx sp., Scatophagus argus* (Linn.), *Arius thalassinus* (Rüppell), *Parachaetodon ocellatus* (Cuvier and Valenciennes), and *Teuthis rostrata* (Cuvier and Valenciennes).

The present paper deals with digenetic trematodes collected during a recent survey of Philippine food fishes. About 35 fishes represented by the above mentioned genera and species of fish yielded 5 genera and species of trematodes belonging to 5 families, of which 3 species are considered new to science. In view of the scanty knowledge of the trematodes of Philippine food fishes it was deemed advisable to describe and make the necessary drawings for all the 5 species of trematodes found.

The fish hosts were bought from Manila markets and vicinity; the name of the locality was supplied by the market vendors except that some specimens of *Scatophagus argus* were collected from the Dagatdagatan Experimental Station through the courtesy of our National Bureau of Fisheries.

The methods used were those reported in my previous papers. All measurements are in millimeters unless otherwise stated; drawings were made with the aid of camera lucida.

Received for publication October 24, 1960.

* Supported in part by a Research Grant (E-2575) from the U.S. National Institutes of Health, U.S. Public Health Service, Bethesda, Md., and a grant-in-aid of the Natural Science Research Center, University of the Philippines.

The author is deeply grateful to Dr. George R. LaRue, Animal Disease and Parasite Research Division, Agricultural Research Center, Beltsville, Maryland, for his encouragement, valuable suggestions and criticisms; and for reading the entire manuscript before it went to press.

Identification of the fish hosts were made by Mr. Agustin F. Umali, Ichthyologist, Philippine National Museum.

MONORCHIIDAE Odhner, 1911

Hurleytrematoides malabonensis n. sp.
(Figs. 7, 8 and 9)

Host: *Parachaetodon ocellatus* (Cuvier and Valenciennes).

Location: Intestine.

Locality: Malabon, Rizal, Luzon Island, Philippines (bought from Central Market, Manila, Philippines).

Prevalence: 11 from 3 hosts.

Type and paratypes: U.S. Natl. Mus. Helm. Coll. No. 39475. University of the Philippines, Dept. of Zoology, Helm. Coll. Nos. 541f; 542f, and 570(1)f.

Diagnosis: (Based on 6 fairly extended gravid specimens). Body fragile, elongate, minutely spined, 0.58 to 1.01 long, 0.16 to 0.25 wide. Oral sucker 0.06 to 0.09 in diameter. Acetabulum at about one-third of body length from anterior end, 0.05 to 0.09 in diameter (in 5 specimens); usually midventral, at times (in type) pushed to right side of body. Prepharynx 0.012 to 0.023 (in 2 specimens), in others not discernible. Pharynx 0.04 to 0.058 long, 0.028 to 0.06 wide. Esophagus 0.07 to 0.12 long. Ceca extending to posterior extremity. Genital pore covered with eggs in very gravid worms. In 2 specimens genital pore at level of acetabulum directed towards lateral side. Feebly developed glandular cells at forebody.

Testis subglobular, 0.16 to 0.23 by 0.092 to 0.16 (in 2 specimens), hardly discernible, Ovary subglobular, faintly stained, 0.056 in diameter in one specimen. Metraterm (in 2 specimens) 0.12 to 0.16 long, 0.023 wide; spination at aperture discernible (fig. 9). Cirrus sac (fig. 9) 0.07 long, 0.023 wide (in 2 specimens): spination of cirrus not seen. Uterus extending to posterior extremity, partly covering testis and ovary in type; in others covering sex organs completely. Vitellaria follicular, irregularly shaped masses in midbody displaced to left, immediately preovarian, confluent medially, dorsal to uterus. Eggs, pear-shaped, operculated, 35 to 46 by 16 to 21 microns, each with an unusually long polar filament about 7 to 8 times length of egg (fig. 8). Excretory bladder saccular; arising from it are 2 broad arms, extent anteriorly not traced.

On the basis of Yamaguti's (1953) key to *Hurleytrema* and allied genera of Monorchiidae, I have assigned the present species to the genus *Hurleytrematoides*.

H. malabonensis is the second species in

the genus. It is distinctly different from *Hurleytrematodies chaetodoni* (Manter, 1942) Yamaguti, 1953, in the character of the vitellaria and the extremely long filament of the egg.

WARETREMATIDAE Srivastava 1939, emended
Syn.: Megasolenidae Yamaguti, 1942
Megasolenidae Skrjabin, 1942

Waretrema piscicola Srivastava, 1939
(Figs. 3, 4, 5 and 6)

Host: *Scatophagus argus* (Linn.).
Location: Intestine.
Locality: Obando, Bulacan, Philippines; Dagatdagatan, Malabon, Rizal, Luzon Island, Philippines.
Prevalence: 5 from 2 of 7 hosts; 26 from 3 of 24 hosts.
Topotypes: U.S. Natl. Mus. Helm. Coll. No. 39476; University of the Philippines, Dept. of Zoology, Helm. Coll. Nos. 666(1)e, and 647(6)e.

This species was collected from several fishes from fish ponds at Obando, Bulacan and Dagatdagatan, Malabon, Rizal, Luzon Island, Philippines.

Descriptive data: (Based on 4 fairly extended gravid specimens.) Body muscular, cylindrical, 1.25 to 3.15 long, 0.24 to 0.53 wide. Oral sucker 0.13 to 0.37 in diameter with dorsal crown of 6 conical papillae. Oral sucker with contracted papillae as shown in figure 5. Prepharynx 0.04 to 0.10 long. Pharynx 0.07 to 0.21 long, 0.09 to 0.16 wide. Esophagus 0.33 to 0.76 long. Acetabulum 0.13 to 0.24 in diameter. Testis single, subglobular to oval, 0.2 to 0.5 long, 0.3 to 0.15 in maximum diameter, appearing at times indented at margin in mid-region due to position of tubular vitellaria. Vesicula seminalis externa 0.08 to 0.12 long, 0.04 to 0.07 wide. Vesicula seminalis interna (one specimen) 0.25 long, 0.09 wide. Pars prostatica coiled (one specimen) 0.6 long, 0.03 wide. Metraterm (one specimen) 0.6 long, 0.08 wide. Hermaphroditic sac 0.22 to 0.69 long, 0.06 to 0.15 wide (fig. 4). Ovary immediately anterior to testis, globular, 0.08 to 0.14 in maximum diameter. Mehlis' gland (one specimen) about size of ovary immediately anterior to latter. Seminal receptacle prominent, posterior to vesicula seminalis externa. Vitellaria 10 large tubules, 5 anterior and 5 posterior. Eggs thin-shelled, 50 to 60 by 20 to 30 microns. Excretory bladder Y-shaped. Excretory pore subterminal. A critical study of one specimen (fig. 6) showed, at the anterior region just below the oral sucker on the two sides, a pair of collecting vessels, which descend posteriorly and lie adjacent to esophagus and the ceca.

Although smaller, in view of the similarities in morphological character and relative measurements, this worm is assigned to *Waretrema piscicola Srivastava*, 1939, from a different host and locality. Upon examination of several specimens, the cuticular spines were found not to be confined to the preacetabular region as claimed by Srivastava (1939) but occurred throughout the entire length of the body, although less prominent posteriorly. In some specimens the spines are lost probably during the preparation of the slides.

It was also noted that the body wall contained peculiar black minute granular deposits of varying sizes which were more numerous anteriorly. Manter (1935) observed similar black granules and collecting tubules in *Megasolena estrix* Linton, 1910.

ANGIODICTYIDAE LOOSS, 1902

Hexangium sigani Goto and Ozaki, 1929
(Figs. 10, 11, 12 and 13)

Host: *Teuthis rostrata* (Cuvier and Valenciennes).
Syn.: *Siganus fuscescens* Seale and Bean.
Location: Intestine.
Locality: Malabon, Rizal, Luzon Island, Philippines; Manila Bay.
Prevalence: Six mature and 7 immature from 3 of 5 hosts.
Topotypes: U.S. Natl. Mus. Helm. Coll. No. 39477; University of the Philippines, Helm. Coll. Nos. 542(1)f2, 549(1)f1, and 551(1)f1.

Although smaller than originally described by Goto and Ozaki (1929), I have referred these worms to their species since they conform to the general morphology and relative body measurements, and fall within the range of egg size. The character and number of longitudinal stems of the lymphatic vessels as described by Yamaguti (1934) were also noted. The posterior branches of the lymphatic vessels are shown in figure 11.

The two Philippine species *Hexangium affinum* Tubangui, 1944, and *Hexangium secundum* Annereaux, 1947, described from single specimens differ from *Hexangium sigani* Goto and Ozaki, 1929, in size and arrangement of the testes. Tubangui's species has almost symmetrical testes and smaller body. *H. secundum* of Annereaux has symmetrical testes, larger ovary, and small body size but larger than Tubangui's species. Variability in the position of the testes (figs. 10, 11, 12 and 13) and discernibility of the cirrus sac were noted in some of the 12 Philippine specimens of *H. sigani*. Cuticular spines are rather minute and their absence in some may be attributed to the processes involved in the preparation of the slides. Except for the arrangement of the testes *H. affinum* and *H. secundum* fall within the range of measurements of 8 of my specimens of *H. sigani* and those of Yamaguti (1953) collected from Celebes, Borneo (table I). The hosts of

TABLE 1. *Comparative measurements of three known species of* Hexangium *Goto and Ozaki, 1929, in millimeters unless otherwise designated.*

	Hexangium sigani Goto and Ozaki, 1929	*Hexangium affinum* Tubangui and Masilungan, 1944	*Hexangium secundum* Annereaux, 1947	*Hexangium sigani* Goto and Ozaki, 1929, by Yamaguti, 1953, from Celebes, Borneo	*Hexangium* sigani* Goto and Ozaki, 1929, by Velasquez
Length of body	8–10	2.7 –3.0	4.17	3.6 –5.3	1.51 –4.5
Width of body	1.8–2.2	0.8 –1.0	1.02	0.7 –1.15	0.31 –1.1
Oral sucker, diameter	0.38–0.45	0.2 –0.24	0.21	0.12–0.13 × 0.13–0.15	0.11 –0.25
Prepharynx, length					
"esophagus" of Yamaguti	1.45–1.65	0.4 –0.46	0.56	0.5 –0.85	0.03 –0.69
Pseudopharynx, length	0.25	0.12–0.15 × 0.12–0.17	0.13		0.06 –0.12
Testes	0.9–1.1 in diameter	0.24–0.26 × 0.24–0.30	0.30 in diameter	0.4 –0.55 × 0.3 –0.45	0.04 –0.4
Ovary	about ⅓ diameter of testis	0.10–0.12 × 0.1 –0.16	0.24	0.2 –0.3 × 0.15–0.25	0.07 –0.28 0.069–0.09 70–80
Cirrus sac, diameter	0.33–0.5	0.10–0.12			
Eggs	77–85 × 50–56 microns	80–100 × 48–57 microns	82–90 × 52–56 microns	75–81 × 54 microns	× 50–60 microns
Host	*Siganus fuscescens*	*Amphacanthus javus*	*Teuthis concatenata* (Cuvier and Valenciennes)	*Siganus* sp.	*Teuthis rostrata* (Cuvier and Valenciennes) Syn. *Siganus fuscescens* Seale and Bean.

* Based on 5 mature and 7 immature specimens.

all the above belong to the Siganidae.

In view of the above facts, I conclude that *H. affinum* and *H. secundum* are objective synonyms of *H. sigani* Goto and Ozaki, 1929.

MONODHELMINTHIDAE Dollfus, 1937

Monodhelmis philippinensis n. sp.
(Fig. 2)

Host: *Arius thalassinus* (Rupell).

Location: Intestine.

Locality: Quiape market, Manila, Philippines.

Prevalence: 13 gravid specimens from 2 hosts.

Type and paratypes: U.S. Nat. Mus. Helm. Coll. No. 39478, University of the Philippines, Dept. of Zoology, Helm. Coll. Nos. 74e; 395(3)f_1.

Diagnosis: (Based on 3 fairly extended specimens.) Body flattened, subcylindrical blunt at both ends, 2.9 to 3.5 long, 0.69 to 0.78 in maximum width. Cuticle thin, spinose. Oral sucker subterminal, 0.35 to 0.36 by 0.33 to 0.36. Prepharynx present, pharynx 0.14 to 0.16 by 0.15 to 0.18, bilobed anteriorly (fig. 2). Esophagus 0.14 to 0.16 by 0.05 to 0.07, bulbous distally. Ceca long and narrow almost reaching posterior extremity. Acetabulum 0.15 to 0.3 by 0.23 to 0.32, weakly muscular, about middle of body posterior to testes.

Testes pear-shaped, anterior end wider, blunt, posterior end narrower, pointed; symmetrical, intercecal, immediately anterior to acetabulum; right testis 0.18 to 0.28 long, 0.092 to 0.15 wide; left testis, 0.2 to 0.32 long, 0.1 to 0.14 wide. Left vas efferens (in type) with a peculiar pyriform, elongated sac adjacent to inner side of left testis. Vesicula seminalis 0.35 to 0.42 long, dilated at proximal end in type. Pars prostatica not clearly discernible. Prostate cells extending all over proximal end of vesicula seminalis and basal portion of accessory reproductive organ. Genital atrium muscular, 0.3 to 0.36 long, 0.23 to 0.25 wide, proximally provided with an accessory organ, cylindrical, flask-shaped with a swollen basal portion made up of two coats, an outer muscular one, 0.12 thick (in type) and a rather thin, less muscular inner layer. Lumen of flask-like structure filled with protoplasmic mass, rather dense at distal end. Anterior portion of flask dome-shaped with a spike-like anterior prostrusible structure. Genital pore mid-ventral at anterior third of body length.

Ovary subglobular 0.12 by 0.092 (in type). Receptaculum seminis prominent; Laurer's canal not seen. Uterine coils occupying entire post-testicular intercecal field; extracecal at posterior region in type. Ascending part of uterus opening into genital pore. Eggs numerous, segmented, not fully embryonated, 58 by 23 to 28 microns extending anteriorly not beyond level of testes and posteriorly about to last fifth of body. Vitelline ducts symmetrical, uniting mid-ventrally to form small vitelline reservoir between testes. Excretory vesicle V-shaped with two longitudinal arms, dorsal, extending anteriorly to posterior margin of oral sucker.

The present species closely resemble *Mono-*

dhelmis arii Yamaguti, 1952, and the fish host belongs to the same genus as Yamaguti's host species. However, *M. philippinensis* must be considered as distinct because it differs from *M. arii* in the large body size, larger and pear-shaped testes, shape of accessory genital organ and extent of vitellaria and excretory arms.

PROSOGONOTREMATIDAE Perez Vigueras, 1940

Prosogonotrema carangi n. sp.
(Fig. 1)

Host: *Caranx* sp.
Location: Stomach.
Locality: Quiapo Market, Manila, Philippines.
Prevalence: One gravid specimen from one host.
Type: U.S. Natl. Mus. Helm. Coll. No. 39479.
Diagnosis: Body muscular, thick, robust, slightly tapering at both ends, 14 in length and 6 in width. Cuticle thick and smooth. Preoral lobes 0.23 thick, with slight median depression presenting slightly bilobed condition. Oral sucker subterminal, 0.83 long, 1.33 wide. Prepharynx and esophagus absent. Pharynx 0.33 long, 0.64 wide. Ceca running transversely from pharynx some distance laterally, turning at right angles, and continuing downward to posterior end of worm. Walls of ceca with irregular folds at anterior end more than at posterior. Acetabulum large, 3.66 by 3.76 located at about three-fourths of body length.

Testes symmetrical, intercecal, at anterior one-fourth of body. Right testis larger than left, 0.97 by 0.62. Left testis globular 0.639 by 0.644. Intertesticular space occupied by convoluted vesicula seminalis and anterior uterine coils. Vesicula seminalis 0.14 to 0.21 wide. Pars prostatica muscular, coiled just posterior to genital atrium. Genital cone not clearly discernible, being covered by eggs in metraterm. Ovary subglobular, 0.69 by 0.41, situated obliquely antero-dextral to acetabutum. Receptaculum seminis ad uterinum just anterior to ovary. Laurer's canal? Mehlis' gland sinistral to ovary, just posterior to receptaculum seminis ad uterinum. Uterine coils mostly intercecal, occupying space anterior to acetabulum, extending laterally to right and left sides of body, then ascending between testes, forming few more coils and finally ending in metraterm, latter extending to a point slightly to left of opening of oral sucker, terminating in cone-shaped structure covered with numerous eggs. Eggs numerous, elliptical, thin, very small, 23 to 28 microns by 7 to 12 microns. Vitellaria consisting of about four loops per side of slender, convoluted, partly branched tubules, extending extracecally; one branch of right gland passing ventral to the mid-ventrolateral margins of the right testis, left gland not extending as far anterior to left testis; stems from two sides uniting with each other at level of oviduct; vitelline reservoir not seen.

Excretory pore dorsal, subterminal, arms of excretory vesicle uniting anteriorly just posterior to pharynx.

In body form this species is somewhat closer to the Celebes species, *Prosogonotrema clupeae* Yamaguti, 1952, except for its large size, slightly bilobed nature of preoral lobes, absence of esophagus, relatively larger acetabulum, more loops in the coils of the vitellaria, and position of Mehlis' gland. The eggs are relatively smaller than in Yamaguti's species and relatively narrower than the Cuban species, *Prosogonotrema bilabiatum* Perez Vigueras, 1940. In view of the above differences *Prosogonotrema carangi* is designated as a distinct species.

SUMMARY

1. Five species of digenetic trematodes belonging to five families, three of them new, are reported from Philippine food fishes.
2. New host and locality records are reported for two previously described species.
3. Variability in the relative position of the testes and discernibility of the cirrus sac were noted in the Philippine specimens of *Hexangium sigani* Goto and Ozaki, 1929, hence these characters are not of specific importance.
4. *Hexangium affinum* Tubangui and Masiluñgan, 1944, and *H. secundum* Annereaux, 1947, are considered objective synonyms of *H. sigani* Goto and Ozaki, 1929, on the basis of the similarities of morphological characters and body measurements.

REFERENCES CITED

GOTO, S. AND OZAKI, Y. 1929 Brief notes on new trematodes. II. Japan. J. Zool. **2**: 369–381.
MANTER, H. W. 1935 The structure and taxonomic position of *Megasolena estrix* Linton 1910 (Trematoda) with notes on related trematodes. Parasit **27**: 431–439.
SRIVASTAVA, H. D. 1939 The morphology and systematic relationships of a new parasite, *Waretrema piscicola* gen. et sp. nov., referrable to a new family—Waretrematidae—of digenetic trematodes. Indian J. Vet. Sci. and An Husb. **9**: 169–172.
TUBANGUI, M. A. 1947 A summary of the parasitic worms from the Philippines. Phil. J. Sci. **76**: 225–322.
YAMAGUTI, S. 1934 Studies on the Helminth Fauna of Japan. Part 2. Trematodes of Fishes. I. Jap. J. Zool. **5**: 249–541.
———— 1953 Parasitic Worms Mainly from Celebes. Part 3. Digenetic trematodes of fishes. II. Acta Med. Okayama **8**: 257–295.

EXPLANATION OF PLATE I

Abbreviations: A, acetabulum; AO, accessory genital organ; EV, excretory vessel; GA, genital atrium; OV, ovary; SR, seminal receptacle; T, testis; VS, seminal vesicle; VT, vitellaria. Unless other wise stated, drawings were made with the aid of camera lucida.

FIGURE 1. Ventral view of *Prosogonotrema carangi* n. sp.

FIGURE 2. Ventral view of *Monodhelmis philippinensis* n. sp.

FIGURE 3. Ventral view of *Waretrema piscicola* Srivastava, 1939.

FIGURE 4. *Waretrema piscicola*, showing terminal portions of reproductive organs.

FIGURE 5. *Waretrema piscicola*, oral sucker with the contracted dorsal crown of papillae.

FIGURE 6. *Waretrema piscicola*, showing the relative position of the collecting tubules. Diagrammatic.

FIGURE 7. *Hurleytrematoides malabonensis* n. sp. Ventral view.

FIGURE 8. *Hurleytrematoides malabonensis*, eggs. Note the very long polar filaments.

FIGURE 9. *Hurleytrematoides malabonensis*, portion showing terminal portion of reproductive organs.

FIGURE 10–13. Posterior portion of four specimens of *Hexangium sigani* Goto and Osaki, 1929, showing the variation in the relative position of the genital organs.

FIGURE 11. *Hexangium sigani*, posterior portion showing the six branches of the lymphatic vessels.

(*Continued from Cover 4*)

DESCRIPTION, LIFE HISTORY, AND DEVELOPMENTAL PATTERN OF *Glypthelmins pennsylvaniensis* N. SP. (TREMATODA: BRACHYCOELIIDAE) NEW PARASITE OF FROGS. **Thomas C. Cheng** (469–477)

STUDIES ON DIGENETIC TREMATODES OF HAWAIIAN FISHES: FAMILY BUCEPHALIDAE. **H. W. Manter and Mary Hanson Pritchard** (479–482)

STUDIES ON DIGENETIC TREMATODES OF HAWAIIAN FISHES: FAMILIES, MONORCHIIDAE AND HAPLOPORIDAE. **H. W. Manter and Mary Hanson Pritchard** (483–492)

DEVELOPMENT OF MOTHER AND DAUGHTER SPOROCYSTS OF *Haplometrana intestinalis* LUCKER, A PLAGIORCHIOID TREMATODE OF FROGS. **Stewart C. Schell** (493–500)

A NEW TREMATODE, *Opisthioglyphe orientalis* N. SP. (PLAGIORCHIIDAE), FROM THE GIANT SALAMANDER IN CHINA. **Han Jong Rim and F. G. Wallace** (501–502)

Macravestibulum kraatzi N. SP. (TREMATODA: PRONOCEPHALIDAE), WITH REVISION OF THE GENUS *Macravestibulum* MACKIN, 1930. **Raymond T. Damian** (503–507)

PROTOPLASMIC PROCESSES OF EPITHELIAL CELLS OF GORGODERA. **G. S. Tulloch, J. E. Shapiro and B. Hershenov** (509–511)

NOTES ON *Sanguinicola davisi* (TREMATODA: SANGUINICOLIDAE) IN THE GILLS OF TROUT. **H. S. Davis, G. L. Hoffman and E. W. Surber** (512–514)

NEW ASPIDOGASTRID TREMATODE, *Cotylaspis reelfootensis*, FROM SOME TENNESSEE MUSSELS. **Haig H. Najarian** (515–520)

SOME DIGENETIC TREMATODES OF PHILIPPINE FOOD FISHES. **Carmen C. Velasquez** (521–526)

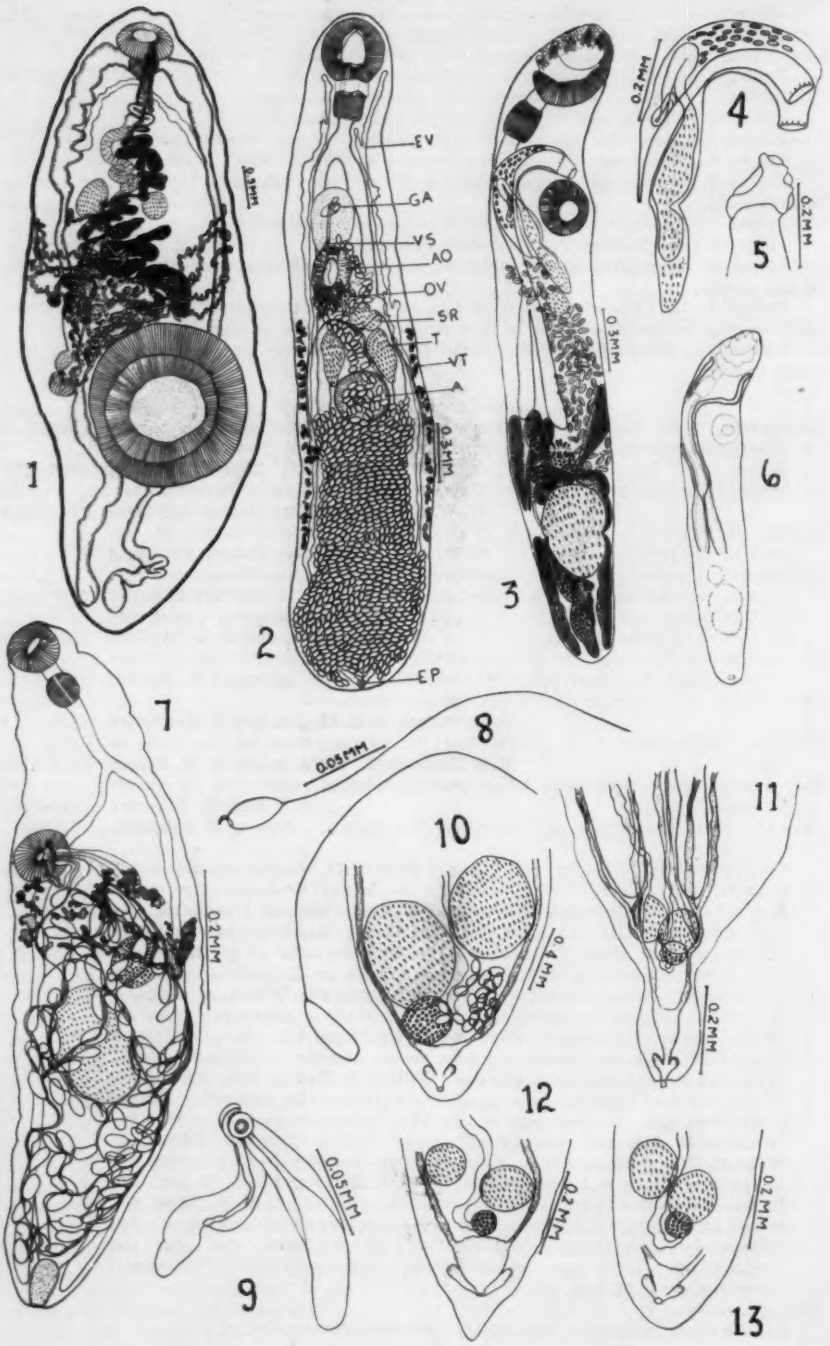
RESEARCH NOTES:

SOME PARASITES OF MAN IN NYASALAND: **Edward D. Wagner and Howard S. Burnett,** 356. EFFECT OF FUMAGILLIN IN MIXED CULTURES CONTAINING *Entamoeba histolytica* AND *Trichomonas hominis:* **Mitsuru Nakamura,** 368. THE DISTRIBUTION OF THE PROGENETIC TREMATODE, *Asymphylodora avicolae* STUNKARD, 1959: **Omer R. Larson,** 371. DEMONSTRATION OF ANTIGEN-ANTIBODY REACTION WITH A MONOBACTERIAL CULTURE OF *Entamoeba histolytica* IN AGAR-GEL AND ON CELLULOSE ACETATE MEMBRANE: **Wasim A. Siddiqui,** 371–372. PARASITIC CERATOPOGONIDAE AND SIMULIIDAE (DIPTERA) FROM IMPERIAL COUNTY, CALIFORNIA: **Raymond E. Ryckman,** 405. A QUANTITATIVE FIELD TECHNIQUE FOR DIFFERENTIATING SOUTH AFRICAN *Schistosoma matteei* CERCARIAE FROM THOSE OF *S. mansoni:* **William B. Rowan,** 406. EMERGENCE OF CERCARIAE OF *Schistosoma mansoni* FROM *Australorbis glabratus:* **Charles S. Richards,** 428. *Cooperia punctata* IN AXIS DEER (*Cervus axis*) IN HAWAII: **Lawrence R. Ash,** 446. SOME PARASITES OF MAN IN ETHIOPIA: **Edward D. Wagner,** 450. SIZE OF ADULT *Nippostrongylus brasiliensis* FROM LIGHT AND HEAVY INFECTIONS IN LABORATORY RATS: **A. J. Haley and J. C. Parker,** 461. IS *Bacillus pinottii* PATHOGENIC IN *Australorbis glabratus?:* **M. R. Tripp,** 464. IS THE CILIATE *Euplotes balteatus* ADAPTING TO COMMENSAL LIFE IN THE SEA URCHIN *Strongylocentrotus dröbachiensis?:* **C. D. Beers,** 478. ON THE ECOLOGY OF *Leidynema appendiculata* (LEIDY, 1850) (NEMATODA: OXYUROIDEA): **C. M. Fay,** 518.

ANNOUNCEMENT:

INFORMATION CONCERNING THE JOURNAL OF PARASITOLOGY, 498.

PLATE I


1
0.1MM
2
EV
GA
VS
AO
OV
SR
T
VT
A
0.1MM
EP
3
0.1MM
4
0.2MM
5
0.2MM
6
7
0.2MM
8
0.09MM
9
0.05MM
10
0.4MM
11
0.2MM
12
0.2MM
13
0.2MM

AMERICAN SOCIETY OF PARASITOLOGISTS

OFFICERS

President: JUSTIN M. ANDREWS
National Institute of Allergy and
Infectious Diseases
National Institutes of Health
Bethesda 14, Maryland

President-Elect: LESLIE A. STAUBER
Department of Zoology
Rutgers University
New Brunswick, New Jersey

Vice-President: GORDON H. BALL
Department of Zoology
University of California
Los Angeles 24, California

Secretary: FRANCIS J. KRUIDENIER
Dept. of Zoology and Physiology
University of Illinois
Urbana, Illinois

Treasurer: MARTIN J. ULMER
Dept. of Zoology and Entomology
Iowa State University
Ames, Iowa

Editor: ELERY R. BECKER
Communicable Disease Center
Public Health Service
702 E. Adams St., Phoenix 34, Ariz.

Custodian: WILFORD S. BAILEY
School of Veterinary Medicine
Auburn University
Auburn, Alabama

COUNCIL MEMBERS AT LARGE

DALE A. PORTER (1958–1961)
Animal Disease Research Laboratory
Agricultural Res. Service, USDA
Auburn, Alabama

A. M. FALLIS (1961)
Ontario Research Foundation
43 Queen's Park
Toronto, Canada

R. V. RINGHAM (1959–1962)
Biology Department
College of Wooster
Wooster, Ohio

L. J. OLIVIER (1959–1962)
Laboratory of Tropical Diseases
National Institutes of Health
Bethesda 14, Maryland

R. M. LEWERT (1960–1963)
Department of Microbiology
University of Chicago
Chicago 37, Illinois

C. P. READ (1960–1963)
Biology Department
Rice Institute
Houston, Texas

R. B. McGHEE (1961–1964)
Department of Biology
University of Georgia
Athens, Georgia

J. A. SCOTT (1961–1964)
Department of Preventative
University of Texas Medical
Galveston, Texas

NON-VOTING REPRESENTATIVES TO COUNCIL

W. E. MARTIN (Southern California Parasitologists)
Department of Zoology
University of Southern California
Los Angeles, California

A. J. HALEY (Helm. Society of Washington)
Department of Zoology
University of Maryland
College Park, Maryland

D. T. CLARK (Annual Midwestern Conference of Parasitologists)
School of Veterinary Medicine
Michigan State University
East Lansing, Michigan

CONTENTS

JOURNAL OF PARASITOLOGY

Volume 47, No. 3 June 1961

THE IN VITRO CULTIVATION OF TAPEWORMS. I. GROWTH OF *Hymenolepis diminuta* (CESTODA: CYCLOPHYLLIDAE). Allen K. Bennett (351–355)

A MECHANICAL APPARATUS FOR SCREENING WORM EGGS FROM FECES. Charles H. Hill and Ralph E. Zimmerman (357–362)

A SIMPLE PROCEDURE FOR RECOVERING SCHISTOSOME EGGS IN MASS FROM TISSUES. Lawrence S. Ritchie and Luis A. Berrios-Duran (363–365)

A PERFUSION PROCEDURE (PERF-O-SUCTION) FOR RECOVERY OF SCHISTOSOME WORMS. Myron G. Radke, L. A. Berrios-Duran and K. Moran (366–368)

A TWO-COMPONENT APPARATUS FOR CONSTRUCTING COBB METAL SLIDES FOR MICROSCOPE MOUNTS. Pierre J. Jutras and A. C. Tarjan (369–371)

PAPER ELECTROPHORESIS OF SERA FROM MAN AND EXPERIMENTAL ANIMALS INFECTED WITH VARIOUS HELMINTHS. Irving G. Kagan and Chauncey G. Goodchild (373–377)

STUDIES ON THE HELMINTHS OF NUTRIA, *Myocastor coypus* (MOLINA), IN LOUISIANA WITH CHECK-LIST OF OTHER WORM PARASITES FROM THIS HOST. Bert B. Babero and J. Warren Lee (378–390)

ENDOPARASITISM IN ISOLATED POPULATIONS OF RODENTS OF THE LAKE BONNEVILLE BASIN, UTAH. John C. Frandsen and Albert W. Grundmann (391–398)

Periglischurus triaenopsis N. SP. PARASITIC ON *Triaenops afer* PETERS (ACARINA: MESOSTIGMATA: SPINTURNICIDAE). P. L. G. Benoit (397–398)

MORTALITY OF EASTERN BROOK TROUT CAUSED BY PLEROCERCOIDS (CESTODA: PSEUDOPHYLLIDAE: DIPHYLLOBOTHRIDAE) IN THE HEART AND VISCERA. Glenn L. Hoffman and C. E. Dunbar (399–400)

CARBOHYDRATE CONTENTS OF THE TAPEWORM *Hymenolepis diminuta* FROM NORMAL, BILATERESS, AND STARVED RATS. Chauncey G. Goodchild (401–405)

SOME PHARMACOLOGICAL ASPECTS OF A TOXIC SUBSTANCE FROM OOCYSTS OF THE COCCIDIUM *Eimeria tenella*. M. T. Rikimaru, F. T. Galysh, and R. F. Shumard (407–412)

STUDIES OF THE AGE AND SEASONAL VARIATIONS IN THE BLOOD AND BONE MARROW PARASITES OF A SERIES OF BLACK-BILLED MAGPIES. Robert M. Stabler (413–416)

THE ENTOCOMMENSAL CILIATE FAUNA OF THE PINK SEA URCHIN, *Allocentrotus fragilis*. Jacques Berger and Robert J. Profant (417–418)

OBSERVATIONS ON THE SPOROGONY AND CHROMOSOME CYCLE OF *Gregarina poecilocerum*. C. C. Narasimhamurti and P. N. Ganapati (419–424)

EFFECT OF SALICYLATES AND RELATED COMPOUNDS ON ANAPHYLACTIC RESPONSE IN RATS INFECTED WITH *Trypanosoma lewisi*. I. ISOMERS OF SALICYLIC ACID. Ellery R. Becker (425–427)

ON THE OCCURRENCE OF *Trichinella spiralis* IN PORK SAUSAGE AVAILABLE IN IOWA. W. J. Zimmermann, L. H. Schwartz and H. E. Biester (429–432)

EFFECT OF SEVERAL NEMATODES ON AN INITIAL INFECTION OF *Nematospiroides dubius* IN MICE. G. Y. H. Lin and M. H. Ivey (433–436)

DESCRIPTION OF *Splendidofilaria flexivaginalis* N. SP. (NEMATODA: ONCHOCERCIDAE) FROM THE EASTERN CROW, AND KEY TO SPECIES OF THE GENUS *Splendidofilaria* SKRJABIN. Joseph Jones, Jr. (437–440)

INCIDENCE OF MICROFILARIAE IN SOME OHIO BIRDS AND DATA ON THE HABITS OF A POSSIBLE VECTOR. Edwin J. Robinson, Jr. (441–444)

EXTENSIVE ABNORMALITIES IN LARVAL TREMATODE INFECTION. Kathleen L. Hussey and Walter R. Stahl (445–446)

Austrobilharzia penneri, A NEW SCHISTOSOME FROM MARINE SNAILS. Robert B. Short and Rhodes B. Hollman (447–452)

CONTRIBUTIONS TO THE LIFE HISTORY OF THE BRAIN FLUKE OF NEWTS AND FISH, *Diplostomulum scheuringi* HUGHES, 1929 (TREMATODA: DIPLOSTOMATIDAE). Frank J. Etges (453–458)

HISTOCHEMICAL GLYCOGEN STUDIES ON *Fasciola hepatica*. Theodor von Brand and Teresa I. Mercado (459–463)

A NEW SPECIES OF *Hysterolecitha* (TREMATODA: HEMIURIDAE) FROM THE MUDSUCKER *Gillichthys mirabilis* COOPER. Roy R. King and Elmer R. Noble (465–468)

(Continued on page 515)

BUSINESS PRESS INC.
Lancaster, Pennsylvania